MYCOLOGIA

OFFICIAL ORGAN OF THE MYCOLOGICAL SOCIETY OF AMERICA

Vol. L MAY-JUNE, 1958 No. 3

CONTENTS

A. J. Mix.....NORVEL M. MCCLUNG 315

The philosophy of taxonomy.....DONALD P. ROGERS 326

Some aspects of morphology, genetics, and cultural behavior of Gelasinospora calospora var. autosteira
EDMUND E. TYLUTKI 333

Taxonomic studies in the Myxomycetes. I. The Trichia favoginea complex.....MARIE L. FARR 357

Antibiotics formed by species of Emericellopsis
F. KAVANAGH, D. TUNIN, AND G. WILD 370

Synchytrium fulgens Schroeter.....JOHN S. KARLING 373

The utilization of oligosaccharides by some species of Ceratocystis.....E. M. WILSON AND VIRGIL GREENE LILLY 376

Studies of a unique pigment complex and a photobiological reaction in Penicillium herquei
J. M. RIEDHART AND C. L. PORTER 390

New water molds from Florida.....A. W. ZIEGLER 403

Studies of some Tremellaceae. II. The genus Ductifera
KENNETH WELLS 407

A preliminary survey of the Gymnoascaceae. I.
HAROLD H. KUEHN 417

Notes and Brief Articles..... 440
Bakerspigel on *Endogone* and *Melanogaster;* Lowy on specimens of fleshy fungi; Brock on heterothallism; medical mycology courses

Reviews..... 447

[MYCOLOGIA for March–April (50: 151–314) was issued June 21, 1958]

PUBLISHED BIMONTHLY FOR

THE NEW YORK BOTANICAL GARDEN

AT PRINCE AND LEMON STS., LANCASTER, PA.

Entered as second-class matter April 20, 1925, at the post office at Lancaster, Pa., under the Act of August 24, 1912.

MYCOLOGIA

Published by

THE NEW YORK BOTANICAL GARDEN

IN COLLABORATION WITH THE

MYCOLOGICAL SOCIETY OF AMERICA

OFFICERS OF THE MYCOLOGICAL SOCIETY OF AMERICA

JOHN R. RAPER, *President*
Harvard University

C. W. EMMONS, *Vice-President*
National Institutes of Health

ALMA WHIFFEN BARKSDALE, *Councilor*, 1958
New York Botanical Garden

C. W. HESSELTINE, *Councilor*, 1958–59
Northern Regional Research and Development Div., U.S.D.A.

C. J. ALEXOPOULOS, *President-elect*
State University of Iowa

E. S. BENEKE, *Sec.-Treas.*
Michigan State University
East Lansing, Michigan

RALPH EMERSON, *Councilor*, 1957–58
University of California

HELEN M. GILKEY, *Councilor*, 1958–59
Oregon State College

EDITORIAL BOARD

DONALD P. ROGERS, *Editor-in-Chief*
Department of Botany
University of Illinois
Urbana, Illinois

CLARK T. ROGERSON, *Managing-Editor*
New York Botanical Garden
Bronx Park, New York 58, N. Y.

H. L. BARNETT, '58
West Virginia University, Morgantown, W. Va.

ALMA WHIFFEN BARKSDALE, '59
New York Botanical Garden,
Bronx Park, New York 58, N. Y.

EDITH K. CASH, '60
Plant Industry Station, Beltsville, Maryland

C. W. EMMONS, '61
National Institutes of Health
Bethesda 14, Maryland

ROY F. CAIN, '62
University of Toronto
Toronto 5, Canada

SUSTAINING MEMBERS

Abbott Laboratories
Baltimore Biological Laboratory, Inc.
Ben Venue Laboratories, Inc.
Buckman Laboratories, Inc.
Ciba Pharmaceutical Products Inc.
Difco Laboratories, Inc.
E. I. DuPont de Nemours & Co.
Lederle Laboratories
Eli Lilly and Company
Ortho Research Foundation
Chas. Pfizer & Co., Inc.
E. R. Squibb and Sons
Standard Brands, Inc.
Arthur H. Thomas Co.

Arthur Jackson Mix, 1888–1956

MYCOLOGIA

OFFICIAL ORGAN OF THE MYCOLOGICAL SOCIETY OF AMERICA

VOL. L MAY–JUNE, 1958 NO. 3

A. J. MIX

NORVEL M. MCCLUNG

(WITH PORTRAIT)

With the death of Professor Arthur Jackson Mix, September 8, 1956, the mycological world lost a devoted friend, enthusiastic scholar, and colorful figure. Although his interests were broad, the fungi held an unfailing fascination for him. His approach to the study and teaching of mycology was classical, sharpened by a discerning knowledge of current mycological research. His earnestness in scholarly pursuits was nicely balanced by a fine awareness of human frailty, especially his own, which a keen sense of humor allowed him to enjoy. He was a world authority on the genus *Taphrina*, a distinction gained by years of careful collecting and research. His interests included the classics and humanities, in which he was well versed. Among his many endowments was a flair for writing amusingly of his experiences so that others could enjoy sharing them. So that he may be allowed to speak for himself on what he chose to call "My Life with *Taphrina*," the other biographical facts will be briefly supplied here. A fuller account will be published in the Transations of the Kansas Academy of Science, volume 61, with a list of his publications.

Arthur Jackson Mix (or A. J., as he preferred to be called) was born in Bolivar, N. Y., September 30, 1888, the youngest of four children of Charles M. and Rose Kenyon Mix. He was graduated from Clinton High School, Clinton, N. Y., in 1906, and completed work for the A.B. degree at Hamilton College in 1910. Following graduation,

[MYCOLOGIA for March–April (**50**: 151–315) was issued June 21, 1958]

he became a fellow at Cornell University in plant pathology, where the Ph.D. degree was granted in 1916. Research for his thesis, entitled "Sun-Scald of Fruit Trees—A Type of Winter Injury," was supervised by Drs. Donald Reddick and H. H. Whetzel. He was assistant botanist at the New York Experimental Station, Geneva, N. Y., the year 1915–16. In the fall of 1916 he was invited by Professor W. C. Stevens to the University of Kansas as instructor in plant pathology, where he rose to the rank of Professor of Botany in 1924, and chairman of the department in 1931. His productive life was spent at the University of Kansas, interrupted by leaves as follows: Assistant Pathologist, U. S. Department of Agriculture, 1917–18; travel, study and collecting in England, Germany, Switzerland and France 1929–30; attending the International Botanical Congress at Cambridge, England, during the summer of 1930; travel and collecting in England, Norway, Sweden, Holland, Belgium, and France and attending the International Botanical Congress at Amsterdam in 1935; travel and collecting in Sweden, Lapland and Norway during the summer of 1939; study at the Farlow Herbarium and teaching at Cornell University, 1939–40; plant pathologist, U. S. Department of Agriculture, 1945–46; travel and collecting in Norway, Sweden, Germany, Italy, France, England, and attending the International Botanical Congress, Stockholm, 1950; travel and collecting in England, Norway, Italy, Canary Islands, Ceylon, India and Japan, 1953–54. For the latter around-the-world trip Prof. Mix was helped by grants from the American Philosophical Society of Philadelphia, and Greater University Fund, University of Kansas; thus at last he made a trip that was not an Unfellowship [1] one.

Professor Mix read Germany, French, Danish, Norwegian, and Swedish with facility and Dutch and Russian with somewhat less ease, and spoke German well. He was also well grounded in both Latin and Greek. Of his many contributions to the Botany Department of K. U., he considered a course in medical mycology which he introduced in 1935 among the most important. At that time only two similar courses were offered in American universities.

In 1917 he married Katherine Lyon, who accompanied him on his many travels and collecting trips. She maintained an active interest in his work and encouraged many friends to enjoy their warm hospitality. The Mix home was a friendly and inviting place visited by many friends from all over the world. The Mixes provided a sanctuary for foreign students, Scandinavians particularly, who attended the University. Prof. and Mrs. Mix frequently attended meetings of the Myco-

[1] See "My Life with *Taphrina*."

logical Society and especially enjoyed the forays. Professor Mix was very capably in charge of the foray at Ithaca, 1952.

It was my privilege and pleasure to be associated with Professor Mix from 1948 until his death. Although he had suffered a mild heart attack in the summer of 1948, he recovered quickly and engaged in a full teaching and research program until his final illness, which began in June of 1956.

His *alma mater,* Hamilton College, in recognition of his many accomplishments awarded him an LL.D. degree in 1953. He held membership in Phi Beta Kappa, Sigma Xi (president, Kansas Chapter, 1950–52), Phi Sigma, the Botanical Society of America, American Phytopathological Society, Mycological Society of America (Councillor, 1948; Vice President, 1952), Kansas Academy of Sciences, Société Linnéenne de Lyon, and the American Association for the Advancement of Science, of which he was a Fellow. He was a member of Alpha Delta Phi, social fraternity, and was listed in Who's Who in America 1954–57. He was the author of some forty scientific papers, and at the time of his death had almost completed the manuscript of Taphrinales for the North American Flora.

The following is from Dr. Mix's presidential address presented to the Kansas Chapter of Sigma Xi in May, 1952:

My Life with Taphrina

We are called companions in zealous research, but ours is not always a close companionship. Sometimes, in intervals between disappearances into our several burrows, we visit with each other. If we try to exchange accounts of our journeys through these burrows some degree of incomprehension is sure to result. The biologist does not always understand the language of the physicist; neither of these may know what a psychologist is talking about. When it comes to our wives, they are often bored by our scientific jargon, and wisely do not attempt to understand it. Ourselves they understand only too well. It has been the custom for your president to speak to the society of his own research. But there are no prescribed rules to follow, nor any established form. I have therefore decided to present something from which the scientific jargon has been eliminated, trying to preserve a nice balance between the inessential and the important. Which is which I leave to you. What follows then, is actually a partial autobiography, the revelation of a hidden life. I have called it "My Life with *Taphrina.*"

I am a mycologist, that is to say a lower variety of botanist; one who studies lower plants: the fungi. Surely you all know fungi. You have seen green molds on oranges; you have eaten a blue mold in Roquefort cheese; you have taken penicillin; if you have made bread, wine or (forgive me) beer, you know how yeasts perform; you have bought mushrooms, perhaps even collected different kinds; if you have looked closely at a wheat field you have seen wheat rust.

Taphrina is the euphonious name of a genus of fungi. This genus contains, according to a recent authority (myself) ninety-nine species; all of them live as parasites on plants ranging in size from the lowly fern to the seed plants. Some cause conspicuous leaf spots, but many induce thickened and distorted leaves, curious galls, striking outgrowths, or close clusters of twigs called witches' brooms. Years ago a stray Japanese student translated for me a paper on cherry witches' broom. His version of the title was: "On the nest of the mountain goblin of the cherry tree"; so *Taphrina* is a witch or at least a mountain goblin, and perhaps I have been bewitched.

My devotion to *Taphrina* began many years ago. I was teaching plant pathology to a single student. She was intelligent but very quiet. In the long laboratory silences I needed employment for my restive mind. Peach leaf-curl became abundant on peach trees west of the University —trees long gone—some of them close to the campus. Peach leaf-curl, brought into the laboratory, kept up both busy.

I watched through the microscope the behavior of the spores of the leaf-curl fungus aptly named *Taphrina deformans.* These spores, I saw, increased in number by a process known as budding, just as yeast cells do. I had been carefully taught. All species of *Taphrina,* I knew, were obligate parasites, and obligate parasites cannot be grown in artificial culture. But yeasts can, and *Taphrina deformans* was behaving like a yeast. So I tried to grow the fungus in culture media, and, *mirabile dictu,* it grew.

I hadn't used the careful technique of isolation that I later learned, and it was apparent that I had cultured more than one fungus. Some cultures died, and finally only one fungus survived. It might or might not be *Taphrina deformans;* how was I to know? Finally, late the following winter, in a ponderous German tome I found in a foot-note a description of the fungus I had been treasuring. Unhappily I labeled my file of careful notes *Pullularia pullulans* (unwelcome name) and waited for another spring.

This time I got the right fungus but it grew so very slowly, feebly, a second summer, fall, and winter were spent trying to liven it up, but

it wouldn't liven. The third year, quite by chance, I put some of my cultures in the refrigerator. Also I forgot them till I had worked long with other laggard cultures. Then I learned the first important fact about the growth of *Taphrina deformans* in culture. Like perspiring K. U. professors, it finds summer heat unpleasant and unfavorable. In fact it will not endure for long temperatures above 85.

The next few years I spent unraveling the life history of the peach leaf-curl fungus, and later, that of the species causing plum pockets. This involved some effort, even the occasional climbing of a peach tree, and the preparation of a report read at a meeting of the American Phytopathological Society. While my paper was in press, a report appeared by Dr. Klebahn of Hamburg, describing cultural studies of several species of *Taphrina*. This, after the first shock, was really gratifying, for Dr. Klebahn was a famous mycologist, renowned for the accuracy of his work.

The next year (1929) I took sabbatical leave and we went to Europe, on an Unguggenheim fellowship. In London I enrolled for the fourth-year curriculum in Botany at the Royal College of Science. I had my cultures with me but I spent little time with them, preferring to study the behavior of other fungi, under the guidance of Professor William Brown, a very capable and friendly man.

We lived in a basement flat in Bloomsbury with a good view of the feet of passers-by. We called our home, with great affection, The Better 'Ole. We explored London and the nearby country on foot and by bus; we queued up for theater seats in the gallery or the pit, and otherwise enjoyed ourselves. During the long Easter vacation we toured England by bus. The hawthorn hedges were in bloom, or, in English parlance, "The may was out," and the weather was gorgeous. It rained only at Stratford-on-Avon, where we stayed at a guest house and found what the English call the w.c. at the end of a long walk under the dripping sky. And we slept in an ancient four-poster which might well have been Shakespeare's second-best bed.

In the summer we went to Hamburg and I worked with Dr. Klebahn, a genial, kindly man who spoke excellent English. We were able, however, to practice our German, for Frau Klebahn, though very friendly, insisted: "Wir müssen immer Deutsch sprechen." The Klebahn son was employed in a linoleum factory in the suburbs. He entertained us at dinner in his bachelor quarters within the factory grounds, behind a great barbed-wire fence well manned by guards. I have often wondered how long it took to convert that factory to munitions or whether it ever produced linoleum at all.

One of Dr. Klebahn's students had discovered that the spores of *Taphrina epiphylla* were wont to conjugate in pairs, a fact of considerable biological significance, if I had time to explain it. I wanted to repeat this work, and Dr. Klebahn took me to *Eppendorfer Moor* and helped me find *Taphrina epiphylla*. As you know, a moor, in German, is a marsh. Besides the alders this moor had many interesting plants, including such insect catchers as the sundew. On my visits there I also saw a bit of German countryside, including a bucolic and alcoholic wedding feast at a nearby *gasthaus*. Or, waiting for the bus, I watched a company of police drilling in a great field across the way. They looked very military in their trim black uniforms and their maneuvers were executed with dash and precision. You recall that at this time Germany was unarmed. I still treasure the permit that allowed me to collect specimens. It reads, "Herr Dr. Mix ist berechtigt, im Eppendorfermoor Zweigteile von Bäumen und Büschen für wissenschaftliche Zwecke abzuschneiden."

In Dr. Klebahn's laboratory I set to work to obtain separate cultures from each of the eight spores contained in a spore-sac of *Taphrina epiphylla*. I had a small home-made micromanipulator devised by Dr. Horr. It was very good, but my luck wasn't. The spore sacs obligingly shot their spores onto agar but they hung together with some sticky epiplasm which hindered my dragging them apart. Afterwards I found such separation of spores much easier with Kansas species.

I couldn't smoke in the laboratory but the Botanic Garden was attractive and at a booth near the entrance luscious sweet cherries were for sale. Homeward bound I rode in an absurd little motor launch across the *Binnen Alster* or walked along the shore, much intrigued by the swan preserve and by the various bathing beaches set apart for adults, for children, for horses and for dogs. At "für Hunden" one could watch the great Doberman Pinschers dash into the water to retrieve sticks. Once a nursemaid who stood in the way was neatly upended into a puddle.

Home at Mundsburgerdamm we didn't like our landlady, nor her moth-eaten furniture, nor the regenegade Irish girl from Brooklyn who worked at the American consulate and bluffed us out of attending the Consul's Fourth-of-July picnic, nor the down puffs under which we tried to sleep, nor waking at dawn to the sound of marching feet and seeing the brownshirts swinging by. But we did like the hardworked maid, Elena, and a foolish little dog called Putzi, and the mug of Pilsener we had with our dinner.

After weeks of arduous micromanipulation, though I had spied upon

the conjugation of spores of *Taphrina epiphylla,* I had to be content with seven single-spore cultures instead of the eight I had hoped for. Actually, seven were enough.

During the summer we went on various excursions, out along the Elbe, to Potsdam and Sans Souci and to Berlin. In August we went by train to Köln and Bonn, by Rhine steamer to Mainz, then to Heidelberg and Rothenburg, to the Passion Play at Oberammergau, through Switzerland and France back to England. At the Cambridge International Botanical Congress we had a reunion with the Klebahns and with English and American friends.

We made a summer trip to Europe again in 1935. Place names have always intrigued me and part of my pleasure in collecting has been in the names of the sources of my specimens. Here are a few from that summer: *Taphrina sadebeckii* at Killarney, *T. betulina* at Killadreenan House, County Wicklow, *T. carpini* at Virginia Water near Windsor Park, *T. ulmi* at Ightham Mote in Kent, *T. tosquinetii* from Trollhättan, Sweden, *T. epiphylla* from Ulvick in Hardangervidda, Norway.

It was in 1935 that I published my summing up of peach leaf-curl, and again, such are the fortunes of research, I was second to a young Canadian who had published his the year before. But our results agreed and mine were the more extensive. Soon afterward fate sent him to work in an irrigated peach-growing area, where, from lack of rain, peach leaf-curl can never grow. I could wish him no sadder lot.

I next undertook to study all species of *Taphrina,* and to make a thorough revision of the genus, looking toward an eventual monograph. This meant obtaining as gifts or as loans specimens from all over the world. It also entailed working in certain museums, as will appear later.

It now became necessary for me to decide which species to leave undisturbed, which to reduce to synonymy with other species, which to describe as new. Very few botanists know what a species is, and so far as I am aware, not a single mycologist does. Some other biologists think they do, but I have ceased to debate with them. In spite of this handicap I boldly attacked the consideration of species. A number had been newly discovered; these could be named and described. A few had been poorly and mistakenly studied. These could be redescribed. But many were very much alike and grew on closely related plants. Dealing with them was difficult. In desperation I once considered reducing all species to synonymy with one well known fungus, called *Taphrina confusa.*

After some years at this engrossing task wanderlust seized us again and in 1939 we went to Europe, on my second sabbatical; this time on

an Unfellowship of the National Academy of Sciences, who said war in Europe was inevitable and immediate and no awards would be made for study abroad. But at this time a celebrated savant, then on the K. U. faculty, a zealous researcher, who could predict the future from the past (to be sure he missed the return of wet seasons to Kansas by several years) assured us that war in Europe would not begin in 1939. So to Europe we went.

We had four days in London, and, working in Professor Brown's laboratory, isolated *Taphrina deformans* from almond leaves picked in late afternoon at Aberdeen and St. Andrews and sent to London by night express. I wanted to know whether the fungi on peach and almond were the same. They are.

From England we crossed the North Sea to Gothenburg on a little freighter named the *Tasso*. The pronunciation should be Tosso. At Stockholm we lived in an apartment on Drottning Kristinasväg and I worked at the Botanical Museum and at Statens Växtskyddsanstalt (National Institute for Plant Protection) a few miles out of the city. Here again I succumb to place names. From that time I have *Taphrina padi* from Experimentalfältet, *T. populi* from Ystad in Skåne, *T. Sadebeckii* from Rydbo, *T. epiphylla* from the Hortus Bergianus or, if you prefer, from Bergianska Trädgården, *T. tosquinetii* from Virabruk in Roslagskulle, *T. betulina* from Tungelsta. Tungelsta is a small village south of Stockholm; we went there in 1939 and again in 1950 in search of a species called *Taphrina lata*, described by an energetic and elusive Swedish mycologist who was good at naming new species, and equally good at losing or mislaying the specimens from which he described them.

When not hunting for these evasive specimens I was looking for the man himself. His fellow Swedes disclaimed knowledge of his whereabouts. It turned out that in 1935 he had been in the near-east, and in 1939 at Düsseldorf. Now he is at Gothenburg and has asked me to drop in. If I do he will probably have just left for Russia.

In August 1939 we went to Lapland, inside the Arctic Circle, which is a neat curving row of small boulders painted white. We stayed at the tourist station at Abisko, located in a National Wildlife Preserve where the birches bristle with witches' brooms, and it is against the law to pick a leaf or twig. Before going I tried to get a permit like the *Ausweis* from Hamburg, but I learned that no such documents were issued, not even to the Swedes themselves. I wanted one species collected at Abisko by a man named Juel and described by him. I have often wondered whether he poached as I did; of course he could have done his collecting outside of the preserve but it is hard to believe.

Anyhow I have one from Abisko, *T. illegalis*. In late August we went to the mountains of Jämtland for more collecting. There we waited for war, reading the London *Times* of the day before, Stockholm morning papers in the evening, and listening to the eight o'clock broadcast, in Swedish. Nothing could be learned from the expressions of the impassive Swedes, and the English-speaking Norwegian pianist was always in a hurry to begin playing for dancing. He would only say: "Nothing has happen, they have had more talks."

With enough of collecting and of apprenhension we returned to Stockholm, where, one pleasant morning the clerk at our hotel exclaimed, "I have just hear on the radio, Hitler have taken Varsava tonight." Though not yet there the Germans were on their way to Warsaw, and after two anxious weeks at Ratvik in beautiful Dalarna, doing more collecting while Hitler marched on Poland, we got passage home, sailing by the *Stavangerfjord* from Oslo.

I had finished with the material at the Stockholm Museum and was able to continue with specimens at the Farlow Herbarium at Harvard, and later at Cornell. Also, the collecting near Ithaca was good.

Some American collecting places have good names too. I have found *Taphrina gracilis* at Labrador Lake, near Ithaca, *T. dearnessii* at Big Bear Lake, Minnesota, *T. americana* at El Vado, Boulder Canyon, Colorado, *T. polystichi* in Nantatala Gorge, North Carolina, *T. higginsii* at Blue Springs Farm, Pine Mountain, Georgia.

On the way back from Georgia we stopped at Lutesville, Missouri, for the rare *Taphrina carveri* on silver maple. Found at Tuskegee, Alabama, by George Washington Carver, and named for him, it was found years later by a farmer at Lutesville and sent to the University of Missouri. Coming from Lutesville my wife yielded up her appendix at Poplar Bluff, and since I still have mine we haven't gone that way again.

My monograph of the genus *Taphrina* was published in 1949 and I am still alive; in fact, acknowledgments received have done much for my stamp collection. I began with 120 species and ended up with 99, including 15 that I have described as new. This procedure you will recognize as orthodox. Be ruthless with other people's species; be bold at describing your own. And expect no mercy from later investigators. But still under the spell of the siren, I did not abandon my efforts; I have kept on. I am trying to learn whether I can distinguish species when growing in culture by their use or refusal of certain food substances—a sort of "tell me what you eat, and I'll tell you what you are" procedure, which has been used successfully with yeasts. Results

should confirm or correct the decisions I have made as to the identity of species as they grow on their hosts. It is interesting that out of this may come the belief that *Taphrina* may be related to the yeasts.

Taphrina has always been a lost lamb among the fungi and few people have even dared to guess what her close relatives are. Now it seems that these second cousins twice removed may be yeasts belonging to the genus *Torulopsis*. For this point of view I have confirmatory evidence and opinion from Dr. Wickerham of the U.S.D.A. yeast laboratory at Peoria.

Reading a paper on the nitrogen nutrition of *Taphrina* became a reason for attending the International Botanical Congress in Stockholm in 1950. We went by the rejuvenated *Stavangerfjord* to Oslo. In Norway we had good collecting. At Kongsvoll, high in the mountains, we stayed at an old coaching inn, frequented by Norwegians, especially old ladies. We began a leisurely ascent of *Knutshö* (or Canute's Height). Every few minutes a Norwegian would steam by; a Norwegian's reaction to a mountain is to get to the top of it at once. Lured by *Taphrina* and by alpine flowers we climbed a long way and when we were above the birches my wife remarked in pleased surprise at our progress. "Just wait," I said, "until one of those old ladies passes us." Almost immediately one did.

After the congress we did better. Going again to Jämtland, we struggled up and down Mt. Åreskutan in the pouring rain; we stayed at the hotel in Storlien where we had enjoyed the "nothing has happen" 1939 broadcast; from the hotel we climbed Stenfjället to timber line, always finding *Taphrina*, always with wet feet, and alternately wet and dry above. This was in company with other botanists. We outdistanced only a plump lady from Palestine and a wife from Copenhagen who wore high heels. But we got to the top and back.

When I first began work with *Taphrina* I felt self-conscious. Why be thus occupied in Kansas with only two species known in the state? Now, as the result of arduous search by myself and Ronald McGregor, who can find anything, that number has been increased to eleven. But along with my more abundant knowledge, species of *Taphrina* have themselves become more abundant in Kansas. By now you must have appreciated the constant devotion between *Taphrina* and myself but it is with mixed feelings that I realize that the cohorts of *Taphrina* are closing in on me.

One species on a common fern was for a long time known only from Indiana, Wisconsin, and Neodesha, Kansas, where it had been found by Dr. W. H. Horr. A few years ago it was collected near Leaven-

worth, then in Baldwin Woods, and most recently at Hole-in-the-Rock. For years the presence in Kansas of a species attacking elms was suspected but not known. In 1948 I found it at Baldwin, in 1949 it was abundant south and west of Lawrence, in 1950 it was within half a mile of the campus. Last year Dr. McClung found it on a tree near the botany greenhouse, and one day, as I parked my car, I saw it on a small red elm growing against the north wall of Snow Hall. "Here it is," I thought; "it can't come closer." But now I know it can come earlier; last year Mr. Shields found it at Twelfth and Oread on May 18. This year I found it at 13th and Oread on May 5.

The species on hard maple was till last summer no nearer than Independence, Kansas. While listening to the dedicatory carillon concert I collected it along west campus road; later in front of Hoch Auditorium and just east of Snow Hall.

Thus, as *Taphrina* closes in, it is time for me to stop. It will be apparent to you that I have devoted many years to the study of one small group of fungi. I have brought many species into culture and studied their biology; I have elucidated certain life-histories; I have observed the behavior of nuclei; I have discovered that curiously some of these fungi actually live in the walls of host cells; I have written a monograph of the genus. I have collected in America and in Europe, but regretfully not as yet in South or Central America, India, Ceylon, the Philippines nor Japan. *Taphrina* and I have become inseparable. When I meet a mycologist he says: "Mix? Oh yes, *Taphrina*," or if European, "Ah, *Taphrecna*." Like many zealous researches I have worked diligently in one very small, neglected corner. Like all zealous researchers I have had a good time. I have enjoyed my life with *Taphrina*.

Department of Bacteriology
University of Georgia
Athens, Georgia

THE PHILOSOPHY OF TAXONOMY [1]

DONALD P. ROGERS

One of the tasks of modern philosophy—some philosophers say the only one—is to evaluate the methods and results of the sciences. In this restricted sense, the philosophy of taxonomy means the philosophical basis of taxonomy, or a critique of taxonomy, or a consideration of the validity of taxonomy. For, be it noted, validity and non-validity are attributes of a science as well as a nomenclature. The philosophy of science has been for decades an important and intensively cultivated field, and the special problems of various sciences, like the general subject, have been given careful and repeated study. But in that part of the literature that I have been able to read—there is more than anyone but a full-time philosopher could cover—I find no consideration of the philosophy of taxonomy. The conclusion is inescapable that taxonomists know a good deal more about philosophy than even the scientific philosophers know about the special problem of taxonomy. And yet there is a problem, and it deserves an answer. If the philosophers will not provide it, the taxonomists must try their hand at the job.

As a matter of fact, entirely satisfactory and reliable work can be done in taxonomy, as in other sciences, without philosophy. But there are times when we need to know what we are doing and why; and then we are faced with the philosophical problem. The nudge toward philosophy may come from within, as it did to one of our ablest associates in this society, who one day looked around his office and his books and said, approximately, "This is a pretty funny job for an able-bodied man." Or it may be external, provided by someone like my former associate Professor Legree, who declared that taxonomy doesn't matter, ecology doesn't matter, physiology doesn't matter, but pathology is different: pathology is important. Or the need for a philosophy may come from the work itself, when we encounter such a general matter as the species problem. Sooner or later we ought to understand what we are doing; we ought to have a philosophy of taxonomy.

I have spoken of the science of taxonomy, just as one might speak of the science of ecology, or of genetics, because the philosophical prob-

[1] Presidential address, presented at the Stanford meeting of the Mycological Society of America, August 27, 1957.

lems are the same whether one is dealing with fungi or bacteria or algae or animals. Taxonomy is the science of classification, and includes not merely the identification of individual organisms, not merely their arrangement in species and genera and more inclusive groups, but the whole basis of comparative morphology and physiology, and the whole framework of phylogenetic hypothesis, on which classification is developed. Ultimately, taxonomy is one sort of synthesis of almost everything that is known about living things.

In philosophical works taxonomy is customarily segregated among the "descriptive sciences" or "non-experimental sciences," and thereby and thenceforth cast into outer darkness and oblivion. The flaw in the reasoning here exhibited is the undervaluing of description as a scientific act, and the traditional but wholly unphilosophical deference to experiment. Under ideal circumstances experiment is observation under conditions allowing manipulation of one factor at a time. It is an important technique in all sciences, including taxonomy. But as a matter of practice there are many things that we want to know that are not subject to investigation by experiment; and as a matter of fact there are many things we do know, as surely as we know anything, that were not and cannot be established by experimental procedures. The sciences of taxonomy, morphology, geology, and astronomy are all, for the most part, descriptive and non-experimental sciences. I am persuaded that they are nevertheless sciences, that they are respectable, and that their conclusions are valid.

We are here concerned with the first of these, taxonomy. It is now appropriate to go back of taxonomy, back of non-experimental sciences in general, to examine the criteria, or the notes (to adopt the language of logic), or the characters (to talk like taxonomists) of a science. Then perhaps we may see how taxonomy stands the test.

Of an infinity of definitions one that seems less unsatisfactory than most is this: A science is an organized body of objective knowledge. For this to have any meaning a second definition is immediately needed, one for objective knowledge. And we may define objective knowledge as "those judgments concerning which universal agreement can be obtained." To the unphilosophical observer of scientific—perhaps especially of taxonomic—controversy it may appear highly improbably that such judgments exist. And yet they do. There is a judgment involved in comparing a barometer with a meter-stick, or a needle with a dial; there is a judgment involved in comparing one plant organ with another; there is a judgment involved in determining the form of a basidium, or that a certain rust fructification is telial. Agreement can

be obtained on all of these matters. And in order that we as students of a science may not give ourselves too much superiority over our non-scientific colleagues, we should note here that the materials of all sciences are judgments, or opinions; that scientists have a problem of knowledge, quite as fundamental as those of poets, psychologists, and theologians. But our foundations are *objective* knowledge, and theirs never, or hardly ever, so. We should note also that among the sciences there can be no pecking-order determined by the notion that I study facts while you, or the speaker in the next room, can deal only with opinions. We all start with judgments; if we are careful, we can all start with those judgments concerning which universal agreement can be obtained.

But facts—that is, judgments—are not enough. Taxonomy is more than Saccardo, just as chemistry is more than Beilstein. A science requires not only objective knowledge, but organization. And the type of organization that is the criterion or note or character of a science is the subsumption of the objective knowledge under a general proposition, or rather under an ascending series of propositions, each successively more generalized. Thus in one experimental science we have observations of the behavior of gases when temperature is varied, when pressure is varied, when volume is varied, generalized and included in the gas laws, and these, and comparable observations and generalizations concerning solutes, combined in a molecular theory of matter. In a non-experimental science we have objective judgments—descriptive judgments—concerning the surface of the earth, subsumed under a series of generalizations that ultimately constitute the science of historical geology, and these, with similar astronomical generalizations, constituting much of cosmology. In mycological taxonomy, observations on the spore-producing organs of a mushroom were generalized in a description of its basidium. Further observations of similar organs increased the generality of the description. Study of rust fungi, of puffballs, of jelly-fungi led to descriptive generalizations concerning other sorts of sporophores; and from all these there was developed a conception of the basidium enlarged to describe all types: the homobasidium, the heterobasidium, and the apobasidium. Similar researches in *Peziza*, and then in other groups, led to a generalization summarized in the concept of the ascus. And finally the two descriptions were further combined in the concept of the cell specialized as the site of karyogamy, meiosis, and spore-formation, the zeugite-gonotokont-sporocyte, and the fungi involved, in the current concept of the Eumycetes.

At this stage perhaps we should remind ourselves that just as scientific facts are only judgments, so scientific generalizations, even when we

call them laws, are only hypotheses. The Newtonian law of gravity is a hypothesis concerning the behavior of matter; it is not certain that the next apple to come loose from the branch will fall to earth, although it is highly probable. An assertion that water in a vessel placed over a flame will boil is a hypothesis, based on innumerable past observations of water in like conditions, and explained by a hypothesis of a higher degree of generalization, that of the molecular structure of matter. The statement that a gill-fungus never previously observed will have basidia —another way of saying that all gill-fungi have basidia—is a hypothesis, and is explained by more general hypotheses: the descriptive hypothesis constituted by the idea of basidiomycetes, and the historical hypothesis constituted by our concepts of phylogeny.

In the experimental and the non-experimental or descriptive sciences, then, the materials are of the same sort—objective knowledge; the organization is of the same sort—a series of general propositions or hypotheses. It follows that the validity is of the same sort.

There are other notes or characters of a science that ought to be considered briefly. One is critical discrimination, a phrase which seems to express the axiom of first-grade arithmetic that you can't add apples to pumpkins. More than one theory of reproduction in the simpler organisms has gone on the rocks because its author equated heterothallism to self-sterility, or to sexual differentiation without the sexes. More than one hypothesis of basidial morphology is rendered vulnerable because of its author's inability to distinguish a sterigma from an epibasidium.

Another note of a science is that it deals not with isolated events, but with classes. A third, and a much more important one, is empirical verification, the possibility of predicting, from the hypotheses of a science, events yet unobserved. We all have heard of the naturalist who, discovering an orchid with a grotesquely long nectar-spur, correctly predicted the ultimate discovery of a moth with an equally long proboscis. This is an ecological prediction; the classical taxonomic empirical verification comes when a transitional organism, which according to a phyletic hypothesis should exist, really turns up. In a minor way this verification took place with the discovery of the genus *Ceratobasidium*, which had to have existed if Neuhoff and the Iowans were right about basidia.[2]

[2] A remarkable example of empirical verification of a taxonomic hypothesis is presented in the discovery by Rollins of *Lesquerella stonensis* (Rhodora **57**: 255–258. 1955). "Working on the hypothesis that the plants of [*L. densipila*] var. *maxima* were of hybrid origin and that an unknown *Lesquerella* was involved in

Another characteristic of the sciences, already mentioned, is that their conclusions are tentative and subject to modification by more precise analysis or by further observation. Briefly, if it isn't tentative it isn't science; mathematics and metaphysics, both of which have been called sciences, are in fact something else by this criterion, as by more important ones. But Newton's formulation of the law of gravity has been shown to be only partially, or approximatelly, correct, and the statement that acidity is an expression of hydrogen-ion concentration to be quite incorrect, and so physics and chemistry retain their standing. So does taxonomy: the Englerian system has been superseded as it classifies the spermatophytes, and the Besseyan system (that of C. E. Bessey, of course) as it deals with the fungi; Neuhoff's hypotheses of basidiomycete phylogeny have required modification, and some day the same will, I hope, be true of Rogers's.

Another assertion about a science, although it repeats some of what has already been said and is not strictly a criterion, seems worth citing. A science includes four types of knowledge: empirical (4 spores, 10 grams, 40%); explained, that is subsumed under a higher generalization (symmetrical basidiospores in Gasteromycetes); anticipated (a new rust will prove to be an obligate parasite); and inferred (the existence and properties of electrons, the existence and properties of common ancestral forms). All these characteristics of the sciences are more or less common knowledge; but that they are common to all sciences is not universally acknowledged, by either philosophers or scientists, and so it has seemed necessary to assert them.

There are, furthermore, two quite false criteria that are often advanced; and it is even more important to discuss these. The first is that scientific generalizations are causal. This is a persistent delusion tacitly held by only some scientists, but explicitly announced by most philosophers who deal with the philosophy of science. This is odd, because philosophers seem to be pretty much in agreement about the meaning of causality. It is no more than a regularly observed concatenation of events. When event A (here "event" is used in the philosopher's sense of phenomenon, or situation, and does not imply motion or change) is regularly followed by event B, we say that A is the cause and B the effect. The fire under the kettle causes the water to boil; nitrogen applied to plants causes vegetative growth. Both these statements are supported by repeated observation and possess a

producing these hybrid plants, [he] sought to find the unknown. *L. stonensis* is that heretofore unknown species." As verification of a phytogeographical hypothesis, *L. lyrata* (Rhodora **57**: 252–255) is almost equally impressive.

high degree of probability, but neither is necessarily true, as is, for example, the statement that $2+2=4$. And neither asserts any more than that event A has regularly been observed to follow event B. Causality, then, is a correlation between successive events, a chronologic relation. The validity of a causal assertion, and its pertinence as a criterion of a science, lies in the correlation, and not in the incidental fact that the events are successive.

The second false criterion is that scientific laws are expressible in formulae. This statement is a truism if it means only that scientific statements can be abbreviated, as in a chemical equation or a floral formula or a phylogenetic diagram. But it is meant to assert much more: that the substance of any science can be stated in quantitative terms and by equations. At least one philosopher will not allow even that a causal relation exists unless events A and B are quantitatively stated. As we and the phytopathologists were told many years ago, it is quite possible not only to carry on scientific research by unfashionable (that is, non-quantitative) techniques, but to express scientific generalizations in non-quantitative terms. Fungus infection of cranberries is light one year and heavy another whether or not percentage of loss is known; and the planets all circle the sun in the same direction, regardless of measurements.

What has been said about the real and false criteria of the sciences adds up, now, to this: The so-called experimental sciences and the so-called descriptive, or non-experimental ones, possess in common all those properties that characterize a science. Whatever we may think about its usefulness, however, we must recognize that the distinction has frequently been made. What is its basis? How do the descriptive sciences, like geology and astronomy and morphology and taxonomy, differ from the more completely experimental ones? In one respect: The generalizations that constitute the descriptive sciences express the correlation existing between simultaneous events. Like causal generalizations, they are summaries of observed habits of nature; the habits are of events occurring at the same time, and not one after the other. In an experimental science it is asserted that when A occurs then B will occur. In a descriptive science it is asserted that when A occurs then B occurs also. If we put the kettle on the fire, the water will boil. If an animal has a notochord, it has gill slits. If a plant has an archegonium, it has an embryo. If a fungus has a dikaryotic mycelium in its life-cyle, it has a zeugite. The degree of probability of each of these statements is about the same. One may have about the same confidence in predicting from any of them. Perhaps the classical example of pre-

diction from a descriptive generalization is found in a story concerning Cuvier, the zoologist. One day the devil appeared in his laboratory, complete with horns, hoof, and tail, and threatened to eat him up. Cuvier said, "Quit bothering me. You're obviously strictly herbivorous. Go on outside and eat grass. You can't eat me"; or at least, words to that effect. Even one who doubts the objective accuracy of some of the details of this narrative must acknowledge the validity of the inference, and thereby the validity of descriptive generalizations. Every time one of us calls a specimen *Agaricus campestris* without examining it microscopically to determine whether it has basidia he makes the same acknowledgment.

What, then, are we doing when we work in taxonomy? The same things as in any other science—making judgments and generalizations based on them. Why do we do this? For the same reason as in any other science, to add to our understanding of nature. What is the validity of our generalizations? Of the same kind as in the other sciences; they are more or less probably true, but never absolutely certain; the degree of probability varies within each science. Why is this not universally recognized? Because of the inherited obsession with causality. What does this amount to? Concern with successive, rather than simultaneous, events. If the pecking-order in the sciences is not to be determined by the extent to which they are experimental, how would we determine it? I am opposed to the existence of a pecking-order; but if we must have one, I suggest that it be determined by the complexity of the phenomena under investigation. Would anyone like to write a structural formula for a basidium?

DEPARTMENT OF BOTANY
UNIVERSITY OF ILLINOIS
URBANA, ILLINOIS

SOME ASPECTS OF MORPHOLOGY, GENETICS, AND CULTURAL BEHAVIOR OF GELASINOSPORA CALOSPORA VAR. AUTOSTEIRA [1]

EDMUND E. TYLUTKI

(WITH 2 FIGURES)

The organism used in this study, *Gelasinospora calospora* (Mouton) Moreau & Moreau var. *autosteira* (Alexopoulos & Sun) Alexopoulos & Sun, is a strictly heterothallic pyrenomycete closely allied to *Neurospora* and *Fimetaria*. Alexopoulos and Sun (**1**) gave this form varietal status so as to separate it from the strictly homothallic *Gelasinospora calospora*, which is practically indistinguishable on a morphological basis. When two isolates of opposite compatibility are mated on a suitable medium, dark, pyriform perithecia containing asci with eight uniseriately arranged ascospores are produced. From the cytological study of ascospore formation made by Sun, Alexopoulos, and Wilson (**38**) it is evident that this linear arrangement of the ascospores indicates the exact order of nuclear movement during meiosis. Therefore, just as in such organisms as *Neurospora sitophila*, *N. crassa* and *Bombardia lunata*, an analysis of the tetrads yields information about the segregation of alleles during meiosis (**10**, **11**, **28**, **44**).

Interest in *Gelasinospora calospora* var. *autosteira* was initiated when it was first described by Alexopoulos and Sun (**1**) in 1950. They removed the spores from two asci and found the compatibility factor segregated at first division. In addition, they found considerable variation in: 1) the intensity of the pigmentation of the mycelium, 2) the length of time required for the development of perithecia, 3) the relative numbers of perithecia produced in matings, 4) the distribution of perithecia in Petri-dish matings, and 5) the fertility of isolates after repeated subculture.

Accordingly, this investigation was undertaken to determine to what extent the environmental culture conditions and genetic segregation con-

[1] Portion of a thesis submitted to the School for Advanced Graduate Studies of Michigan State University of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of Doctor of Philosophy, December, 1955.

tributed to the variations mentioned above. This report is also concerned with a description of some additional morphological findings and a further characterization of the mating-type locus.

MATERIALS AND METHODS

DESCRIPTION OF STOCK. The stock of *Gelasinospora calospora* var. *autosteira* employed is that described by Alexopoulos and Sun (**1**) as having been developed in a moist chamber on Spanish moss collected in Natchez, Mississippi, in 1949. All cultures were obtained from single ascospores obtained by mating descendants of some of the original isolates made by Alexopoulos and were maintained on Difco corn meal agar slants stored in a refrigerator at approximately 6° C. Subcultures were established by transferring blocks of agar which contained mycelium to fresh agar slants. This was the only method available as conidia are not produced.

Two single-ascosporic isolates arbitrarily designated a-12 and A-3 were selected as the parents for the matings reported in this investigation. Another isolate, 207.1.5, obtained as an F_1 segregant from the cross a-12 × A-3 was employed in the analysis as a tester strain for mating-type determination.

CULTURE CONDITIONS. Except where stated, the following standard culture conditions were employed: 1) Medium: Difco corn-meal agar slanted in test tubes or 20 ml per Petri dish; 2) Temperature: 20° C obtained with the use of a B.O.D. temperature-control cabinet; 3) Light: artificial and intermittent, supplied by a Sylvania 14-watt cool-light standard fluorescent tube placed at an average distance of 28 cm from the culture level in the incubator. The light was automatically controlled to give 15 hours of light and 9 hours of darkness during each 24 hour period.

TECHNIQUES EMPLOYED IN TETRAD ANALYSIS. The most satisfactory perithecia for the dissection of asci were obtained from cultures (of cross A-3 × a-12) incubated at standard culture conditions for 25 days. After that period, the ripe ascospores were ejected daily in great numbers and the material became progressively less suitable for study.

The individual asci and ascospores were dissected free-hand, employing fine glass needles and magnifications of 45 × and 90 × obtained with a binocular dissecting microscope. Care was taken to retain the exact order of the ascospores as they were removed from the ascus, but orientation of the spores with respect to the top and bottom of the ascus was disregarded. To stimulate germination, the spores were placed on corn-meal-agar medium containing 1:100,000 distilled furfural and heat

treated at 60° C for one-half hour. Thus, by employing a combination of the furfural-stimulation technique of Emmerson (**23**) and the heat-treatment method of Dodge (**11**) excellent germination was obtained. After stimulation and germination the sporelings were cut out and transferred to corn-meal-agar slants for incubation.

For mating-type determinations, the F_1 serial isolates were back-crossed to the parent isolates A-3 and a-12 on agar in Petri dishes. In some instances a negative reaction was obtained and accordingly in those cases it was necessary to resort to an additional mating to F_1 segregant 207.1.5 of mating type Mt^-. The genetic data were analyzed according to the method of Lindegren (**28**) as amplified by Catcheside (**10**). The distance from locus to centromere was determined by one-half the percent of second-division crossovers, uncorrected for double crossovers.

RESULTS AND OBSERVATIONS

MORPHOLOGY. The initial observations of Alexopoulos and Sun (**1**) on the morphology of *Gelasinospora calospora* var. *autosteira* (*G. autosteira*) have been verified throughout this study. However, further investigation has yielded additional information, particularly with regard to the formation of spermatia and receptive hyphae. Although the facts concerning cell fusions and nuclear migrations have not been investigated, from the morphological study presented here it seems likely that a trichogyne-spermatium relationship exists in *Gelasinospora calospora* var. *autosteira*.[2]

When single ascospores are isolated in separate test tubes or Petri dishes and the mycelium is allowed to mature, some of the cultures produce minute conidium-like structures, which in their morphology are similar to the spermatia and spermatiophores of Cain's *Gelasinospora adjuncta* (**9**). Furthermore, since their morphology and manner of formation are similar to those of other pyrenomycetes which are known to produce spermatia (**6**, **16**), it is suggested that these structures be designated spermatia and spermatiophores. Further evidence, although admittedly circumstantial, in support of this view is afforded by the fact that it has been difficult to germinate the small cells to produce mycelia on various media and in hanging drop cultures. In general, these spores are unable to reproduce the organism as readily as can the microconidia of *Neurospora* (**13**).

As the spermatiophores and spermatia are morphologically well de-

[2] See Sloan, B. J., and G. B. Wilson. The functions of the microspores of *Gelasinospora calospora* var. *autosteira*. Mycologia **50**: 111–116. 1958.

fined and constitute a part of the specific characteristics, the original description of *Gelasinospora calospora* var. *autosteira* (**1**) is amended to include the following information: The spermatia are produced on aerial spermatiophores 90–115 μ high, which by their method of branching assume a dendroid appearance (as shown in FIG. 1A). The branches

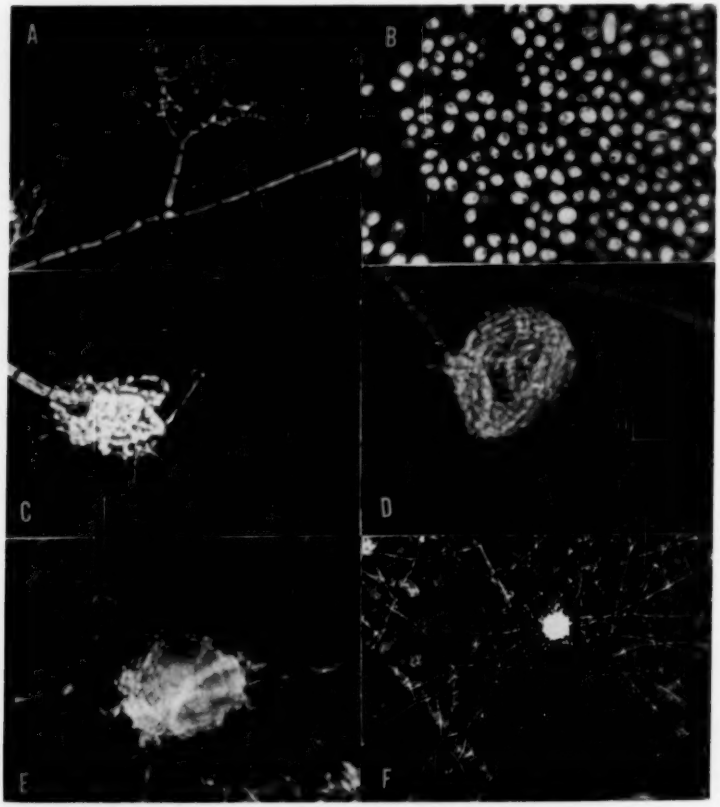


FIG. 1, A–F. Stages of spermatium and protoperithecium formation in *G. calospora* var. *autosteira*. A. Typical dendroid spermatiophore with spermatia *ca.* × 400. B. Spermatia *ca.* × 1000. C. Young protoperithecium with branched trichogyne *ca.* × 440. D. Protoperithecium with unbranched trichogyne *ca.* × 440. E. A mature protoperithecium with enlarged coiled ascogonium surrounded by a pseudo-parenchymatous wall with numerous sterile hyphae *ca.* × 440. F. A protoperithecium with dichotomously branched trichogyne which may be seen extending outward toward the lower left hand corner and the letter "F" *ca.* × 100.

of the spermatiophores consist of short cells, $6 \times 12\,\mu$, each of which bears a single projection from which the spermatia are budded off. These cells, all of which are potentially spermogonia, are more densely protoplasmic and stain darker than the basal, much larger segments of the spermatiophore.

The spermatia do not appear to be organized endogenously, but are budded off one at a time from the projections on the short cells of the spermatiophores. In the first stages of development the spermatia may be observed as subglobose enlargements at the ends of the projections. Later the buds become constricted at the base as development proceeds until, finally, they enlarge into globose cells which are released and pushed to the side as other buds develop from below. After their formation the spermatia may form small clusters about their point of origin. When placed in water for observation, such clusters readily break apart. The globose to subglobose spermatia (FIG. 1B) average $3.5\,\mu$ in diameter. Variation in size and shape may be encountered on some occasions.

On Difco corn-meal agar or Spanish-moss agar (**37**) the individual spermatiophores may arise directly from ordinary hyphae on the surface of the agar or they may be produced in clusters near the terminal portions of specialized stout hyphae which grow up and intermingle with sterile aerial mycelium. These hyphae may often reach a length of 1 mm or more. In some isolates a cushion-shaped spermodochium may be formed, particularly near the edge of the colony or on the glass walls of the culture vessel. In test-tube cultures the spermatiophores may be scattered over the entire slant or, as more often happens, confined to the upper edge of the agar slant where the agar tends to dry out rapidly.

A general account of the formation of protoperithecia was given by Sun (**37**) and published by Alexopoulos and Sun (**1**). However, receptive hyphae have not been reported. In view of the fact that spermatia were found to be produced by this organism, developing protoperithecia were examined rather critically for the presence of receptive hyphae, and it was found that there are produced certain structures which morphologically may be interpreted as trichogynes. However, actual cell fusions involving trichogynes have not been observed.

The trichogyne is best distinguished before the protoperithecium has matured. In early stages of protoperithecial development the ascogonium develops as a terminal or lateral coil which very soon in its development is enveloped by adjoining hyphae to form a young protoperithecium. It is at this young stage, before the pseudoparenchymatous wall is formed with its numerous sterile hyphae arising from the outer edge, that certain long flexuous hyphae may first be seen arising from

within this mass of knotted hyphae. Such trichogynes, shown in FIGS. 1C and D, are slender and septate. They arise internally, presumably from the ascogonium, and extend outward, where they may be observed to branch dichotomously, particularly as they elongate (FIG. 1F). It was not possible to determine with certainty the attachment of the trichogyne to the ascogone for, at early stages of development, the ascogone is not differentiated from the other hyphae within the protoperithecium (FIGS. 1C and D). When the protoperithecium matures the ascogone is plainly visible and assumes the characteristic enlarged coiled shape shown in FIG. 1E. However, at this stage, study of the trichogynes is made most difficult by the many sterile hyphae arising from the outer layer of the pseudoparenchymatous wall.

On the basis of the following morphological distinctions, it is suggested that such long flexuous hyphae are analogous to the trichogynes of other ascomycetes and may function in the development of a protoperithecium into a perithecium:

(1) The trichogynes are more densely stained with cotton blue, especially when the stain is first applied. This same rapid staining of the trichogynes was noted by Dodge (**14**) for *Neurospora sitophila*.
(2) They originate from within the protoperithecium and not from the outer layer as is the case with the other hyphae.
(3) Their method of branching is characteristically dichotomous, thus differing from the random method of branching observed in other hyphae.

It is evident from this study that *Gelasinospora calospora* var. *autosteira* is basically an hermaphroditic organism, for it can be demonstrated that single-ascosporic isolates of both compatibility groups are equally capable of producing spermatiophores and protoperithecia. However, it will be shown later that certain isolates producing only protoperithecia or spermatia may be encountered.

ENVIRONMENTAL STUDIES

Experiments on culture conditions preceded the genetic analysis and were conducted to determine: 1) the optimum conditions for growth and reproduction, 2) to what extent such variable characters as pigmentation, protoperithecium formation and spermatiophore production were influenced by the culture conditions, and 3) the relationship between the environment and certain perithecial distribution patterns obtained in Petri-dish cultures. Of those environmental factors studied, tempera-

ture, light, and substratum were found to be most important in these respects.

Temperature. To demonstrate the influence of temperature on reproduction and general cultural characteristics of *G. calospora* var. *autosteira* the following experiment is described:

The two isolates a-12 and A-3, which normally produce perithecia when mated, were inoculated opposite each other and singly in Petri dishes. Six dishes of each: 1) a-12 and A-3 paired, 2) a-12 grown singly, and 3) A-3 grown singly, were cultured at 12, 16, 20, 24, 28, 30 and 37° C in incubators wherein light was not controlled. Because it was physically impossible to control the light in the incubators available, it was thought that additional information could be obtained by incubating some cultures at room temperature (24–26°) on a laboratory shelf provided with light as described under light control in Materials and Methods.

Aside from noticeable differences in growth rate, the organisms grew at all temperatures tested. Growth was most rapid at 30°, at which temperature after 36 hours the mycelium had completely covered the surface of the agar plates. Cultures incubated at lower temperatures 20, 16, and 12° produced consistently more aerial mycelium than those grown at the higher temperatures.

When supplementary light was provided, young perithecia were observed in three days in those cultures in which A-3 and a-12 were mated and incubated at 24–26°. When such light was not provided, perithecia did not appear until after 6 days in cultures incubated at 24, 28, and 30°, after 9 days in cultures incubated at 20 and 16°, and after 21 days in those incubated at 12°. At 37° growth consisted of vegetative mycelium only, there being no evidence of protoperithecia or perithecia in any of the cultures. Only those ascocarps with beaks as noted with 95 × magnification of the dissecting microscope were judged as perithecia regardless of whether mature ascospores had yet developed.

Information regarding the relationship between temperature and fertility was obtained by counting the number of perithecia produced. TABLE I lists the totals obtained for six Petri dishes at each of the eight temperatures employed. It is of interest to note that although most perithecia were produced in cultures incubated at 24–26° with supplementary light, ascospores were most abundantly produced at 20° with occasional light.

The cultures were also examined for changes in perithecial distribution patterns. Isolates a-12 and A-3 when mated opposite each other in a Petri dish at standard conditions normally produce perithecia dis-

TABLE I

EFFECT OF TEMPERATURE ON THE NUMBER OF PERITHECIA AND THE ABUNDANCE OF ASCOSPORES PRODUCED

Temperature of incubation	Total number of perithecia counted in six petri dishes	Ascospore production
12° C	0	None
16° C	966	Poor
20° C	858	Good
24° C	811	Poor
28° C	128	Poor
30° C	376	None
37° C	0	None
24–26° C*	1,326	Fair
Light controlled		

* Light was controlled in these cultures as explained in the text.
Incubation periods two weeks for perithecial determination; four weeks for ascospore determination.

tributed in a scattered fashion on the a-12 side of the culture only. This pattern is shown in FIG. 2A and is designated Type I.

The temperature at which cultures were incubated modified the perithecial distribution pattern. It was noticed that at 28 and 30° the perithecia showed a marked tendency to form clumps. At 24–26° with supplementary light, the perithecia were produced in the region where

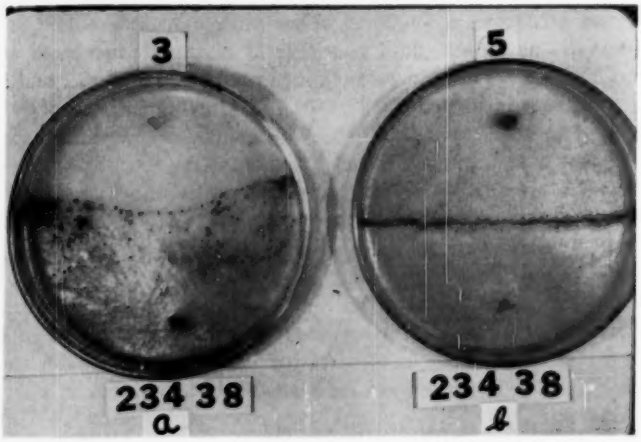


FIG. 2. Two basic perithecial distribution patterns. A. Type I with perithecia scattered on one side. B. Type II with perithecia on both sides forming a line or band.

the two isolates met. However, in all cultures the perithecia were consistently formed on the a-12 side of the culture, no change from this pattern being observed.

Those cultures in which A-3 and a-12 were grown separately exhibited the following: Isolate A-3, which had never been observed to produce protoperithecia throughout the entire investigation, likewise produced no protoperithecia under the conditions of this experiment. Isolate a-12, which normally produced protoperithecia and spermatiophores, formed neither spermatiophores nor protoperithecia in cultures incubated at the two extremes 12° and 37°. In the median temperature range both structures were produced. No noticeable difference was observed between those cultures incubated with or without supplementary light.

Light. Preliminary studies with light, which were conducted during temperature studies, yielded some information regarding the action of light on *G. calospora* var. *autosteira.* However, to demonstrate further the importance of light in the fruiting process of this fungus the following experiment is pertinent:

Random monospore isolates 2–5 and 3–R–25, which normally produced perithecia on the 2–5 side of the culture when mated, were inoculated opposite each other in Petri dishes and incubated at room temperature (24–26°). Five dishes so inoculated were cultured in a cabinet which had been sealed to exclude light. To serve as controls five other plates were placed in a similar locker, and exposed to diffuse daylight for at least 15 minutes each day throughout the culture period.

After 14 days a dish was removed from that group grown in the dark and compared to the control cultures incubated with some light. This comparison revealed that the culture from the dark had neither protoperithecia nor perithecia and, in addition, the pigmentation was more pronounced than in the cultures incubated in the light. The control cultures produced perithecia abundantly and consistently on the 2–5 side. A second dish was removed from the dark after 19 days of incubation and the same results were obtained as noted in the first dish. After 28 days the remaining three dishes were removed from the dark. In two of these last three dishes the reaction was similar to that in cultures removed after 14 and 19 days. However, in one plate several protoperithecia and two perithecia were observed. The perithecia appeared to be normal and contained some mature ascospores.

Pigmentation and mycelial growth differed in the cultures incubated under the two conditions. Although in the dark both strains produced a dark green pigment, strain 2–5 was most heavily pigmented. In the

cultures incubated with occasional light, pigmentation was not as pronounced; a green pigment was noted in isolate 2–5 but not easily detected in strain 3–R–25. The mycelium of cultures incubated in the dark was rather uniformly distributed in the plates so that no line indicating the place where the two isolates had met was discernible, and there was a general tendency for more aerial mycelium to be produced. In cultures grown with some available light there was little or no aerial mycelium and the area at which isolates 2–5 and 3–R–25 met was easily noted.

Cultures incubated in the dark were removed and placed in the light for an additional incubation period. After four or five days protoperithecia and spermatiophores became evident, and upon continued incubation, perithecia were produced in abundance completely scattered over the plates and not restricted to the 2–5 side of the cultures as in the controls. Eventually mature ascospores were obtained.

In another experiment isolates a-12 and A-3 were inoculated on Difco corn-meal agar slants in 75 ml culture tubes. Six of the tubes were coated with black paint to exclude light, and six other tubes were left uncoated as controls. The cultures were incubated at standard culture conditions for three weeks. The same results were obtained as in the previous experiment. Few or no perithecia were formed when culturing was done in the dark, whereas abundant perithecia were formed when light was available during the culture period.

Media. Gelasinospora calospora var. *autosteira* was grown on such different media as potato dextrose agar, malt agar, Czapek's-solution agar, dung agar, Westergaard and Mitchell medium (**39**), Spanish-moss agar, and Difco corn-meal agar. The organism grew well and fruited on most media. However, Difco corn-meal agar proved to be the most satisfactory for culture of this fungus and was used extensively because of the following: 1) substantial growth and sporulation were obtained on this medium; 2) little or no aerial mycelium was produced, and 3) pigmentation of the mycelium which obscured the study of morphological structures was reduced to a minimum.

Since extensive experimentation was conducted in standard Petri dishes, it was thought desirable to determine the optimum quantity of agar to be employed in such cultures. The results are summarized in TABLE II. Excluding those cultures which were sealed, the number of perithecia produced increased with the increased amounts of medium employed, to a maximum at 50 ml. When small quantities of medium were used, fewer larger perithecia were formed and the rate of maturation of perithecia was more rapid than when greater amounts were used.

Ascospore production increased somewhat with increased amounts of medium up to a certain point. At the level of 50 ml of medium per plate, ascospores were not observed in 28 days because the perithecia had not yet matured.

TABLE II

EFFECT OF DEPTH OF CULTURE MEDIUM ON PERITHECIUM FORMATION IN PETRI-DISH MATINGS

Amount of agar per plate	Plate No.	Number of perithecia	Total of perithecia in first 3 plates	Ascospores
10 ml	1	75*		±
	2	161*	306	±
	3	70*		±
	4	41*		±
15 ml	1	156		+
	2	128	415	+
	3	131		+
	4	20*		−
20 ml	1	122		+
	2	154	429	+
	3	153		+
	4	84*		−
25 ml	1	267		+
	2	226	708	+
	3	215		+
	4	116*		−
30 ml	1	215		++
	2	245	699	++
	3	239		++
	4	111*		−
35 ml	1	336		++
	2	262	857	++
	3	259		++
	4	8*		−
40 ml	1	289		++
	2	243	837	++
	3	305		++
	4	237*		+
45 ml	1	417		++
	2	512	1,352	++
	3	423		++
	4	32*		−
50 ml	1	473		−
	2	540	1,584	−
	3	571		−
	4	143*		−

* Cultures were sealed by taping the edge of the dish with masking tape. Incubation at 24° C for 28 days, light not controlled.

The results obtained from the sealed plates differed markedly from the others. The numbers of perithecia produced in all these dishes were consistently lower than in the unsealed dishes. Furthermore, mature ascospores were either rare or absent in most plates.

When isolates a-12 and A-3 were grown separately in Petri dishes containing different amounts of medium, isolate A-3 produced spermatiophores but no protoperithecia. Isolate a-12, however, produced spermatiophores and protoperithecia in all plates except in those with 10 ml of medium. A comparison with the results in TABLE II reveals a strong correlation between the quantity of protoperithecia produced by isolate a-12 and the quantity of perithecia produced on the a-12 side of matings (a-12 × A-3).

GENETIC STUDIES

A preliminary study of approximately 200 random single-ascosporic isolates and monospore cultures derived from the serial dissection of 18 asci revealed that the following characters were segregating: 1) pigmentation of the mycelium, 2) production of protoperithecia, 3) formation of spermatia, and 4) the production of aerial mycelium. Not all the characteristics just mentioned proved to be suitable for genetic analysis within the limits of available time and facilities.

The genetic information presented in this report was accumulated from the single cross (a-12 × A-3). It may be recalled that these parent isolates, a-12 and A-3, selected for tetrad analysis, are the same which had been employed extensively in the environmental studies. All culturing for genetic analysis was conducted under standard culture conditions, which are reported in Materials and Methods. Likewise, the reader is referred to Materials and Methods for details regarding the techniques employed. Mating type was determined by backcrossing the F_1 segregants to the parents A-3 and a-12. Isolate 207.1.5, selected from the F_1, was also employed particularly when a segregant reacted negatively with both a-12 and A-3.

One hundred and forty-two asci were dissected and analyzed. From the segregation patterns obtained it became apparent that the pigmentation of the mycelium was affected by more than one gene. One of the genes studied was Green (G), a factor which imparts a green color to the mycelium in cultures incubated at standard culture conditions for 6–8 days. The length of time the cultures are incubated is important, for in some cultures, beginning after 6–8 days segregation for G is obscured by secondary pigmentation reactions, which, as yet, have not been analyzed. Of the total of 142 asci studied, 112 showed first-division, and 30 second-division segregation for G.

As in most other Ascomycetes the compatibility reaction has been found to be controlled by a single factor pair. Mutations at the Mt locus were not encountered in this study. When both Mt and G are

considered simultaneously, nine segregation patterns were obtained. If the genotype of the parents employed is designated as follows: a-12 = Mt^-G^+; A-3 = Mt^+G, then the genotypes and arrangement of ascospores in asci derived from this cross may be tabulated as in TABLE III. Since adjacent spore pairs in an ascus are genetically identical, the genotypes of the asci are simply represented by tabulations of spore pairs rather than individual spores.

Linkage is indicated by the large number, 112, of asci obtained in which the ascospores are genotypically like the parents. However, linkage may be more readily demonstrated if the patterns of ascus segregation are grouped into ascus-segregation classes (Catcheside, **10**). TABLE

TABLE III

GENOTYPE AND ARRANGEMENT OF ASCOSPORES IN ASCI DERIVED FROM CROSS: $Mt^+G \times Mt^-G^+$

Arrangement	Mt^+G Mt^+G Mt^-G^+ Mt^-G^+	Mt^+G Mt^+G^+ Mt^-G^+ Mt^-G	Mt^+G^+ Mt^+G Mt^-G Mt^-G^+	Mt^+G Mt^+G^+ Mt^-G Mt^-G^+	Mt^+G^+ Mt^+G Mt^-G^+ Mt^-G
No. of asci observed	112	7	6	5	5
Arrangement	Mt^+G^+ Mt^-G^+ Mt^+G Mt^-G	Mt^+G Mt^-G^+ Mt^+G Mt^-G^+	Mt^+G Mt^-G^+ Mt^-G^+ Mt^+G	Mt^+G^+ Mt^-G Mt^+G Mt^-G^+	
No. of asci observed	1	4	1	1	

IV shows the seven segregation classes obtainable for two factors segregating independently. Such a grouping disregards: 1) orientation with respect to base and apex of the ascus, and 2) the way segregations are directed in the second division spindles with respect to one another.

The observed segregation patterns shown in TABLE III were grouped into the appropriate segregation classes shown in TABLE IV. That the G and Mt loci are linked is indicated by the large disparity between segregation classes 1 and 2. If the loci were on separate chromosomes we should expect these two classes to appear with equal frequency. Additional evidence for linkage is found in the high frequency of asci in class 6 where a crossover had occurred but the spore pairs still have a composition like the parents.

Second-division segregation of the G locus and of the Mt locus occurred in 30 and 7 asci respectively. On this basis the calculated recombination between the loci and centromere (one-half second-division-segregation percentage) is 2.4 for Mt, and 10.5 for the G locus. The recombination between Mt and G loci as calculated by determining the

TABLE IV

SEGREGATION CLASSES AND FREQUENCY OF ASCI OBTAINED IN EACH CLASS CROSS: A-12 (Mt^-G^+) × A-3 (Mt^+G)

Segregation classes*	1 Mt^+G^+ Mt^+G^+ Mt^-G Mt^-G	2 Mt^+G Mt^+G Mt^-G^+ Mt^-G^+	3 Mt^+G^+ Mt^+G Mt^-G^+ Mt^-G	4 Mt^+G^+ Mt^-G^+ Mt^+G Mt^-G	5 Mt^+G^+ Mt^-G Mt^+G^+ Mt^-G	6 Mt^+G Mt^-G^+ Mt^+G Mt^-G^+	7 Mt^+G^+ Mt^-G Mt^+G Mt^-G^+	Total
Asci	0	112	23	1	0	5	1	142
2nd div. seg. for Mt	0	0	0	1	0	5	1	7
2nd div. seg. for G	0	0	23	1	0	5	1	30
Recombinant spore pairs	0	0	46	2	0	0	2	50

* Catcheside (10).

percent of recombinant spore pairs is 8.8 Since Mt and G are linked, two relationships are equally possible as regards the relative position of the loci on the chromosome. They may be 1) in the same arm of the chromosome, or 2) in the opposite arms of the chromosome. From TABLE IV this relationship is determined in the following way: If the genes are in opposite arms of the chromosome then the recombination between them, 8.8, should be more nearly the sum, 12.9, of the recombinations between the respective loci and the centromere. If the loci are in the same arm of the chromosome the recombination between them, 8.8, will be more nearly equal to 8.1, the difference of the recombination values between the loci and the centromere. Referring to

TABLE V

RECOMBINATION DATA AND CHROMOSOME MAP

One-half 2nd div. seg. for Mt %	One-half 2nd div. seg. for G %	Total spore pairs	Recombinant spore pairs Mt − G		A + B	A − B
			Total	%		
2.4	10.5	568	50	8.8	12.9	8.1

TABLE V we note that the latter case applies and hence the loci are established as being in the same arm of the chromosome.

Using the recombination values, a chromosome map, uncorrected for double crossovers, may be constructed as shown in TABLE V. The mating-type locus is established as 2.4 units from the centromere and the G locus as 8.8 units from the mating-type locus, as judged by the recombination between the two loci.

Segregation for protoperithecia was observed, but it was not as well defined as the 1:1 segregation obtained for both the Mt and G loci. When the 142 asci analyzed were studied for the production of protoperithecia, the ascal segregation patterns as judged with the unaided eye fell into five groups:

(1) Segregation patterns which showed a 1:1 segregation ratio for protoperithecia. Eighty-four asci were of this type, of which 76 showed first-division segregation and 8 second-division segregation.
(2) Segregation patterns in which none of the isolates produced protoperithecia. Thirty asci were of this type.
(3) Segregation patterns in which all eight isolates produced protoperithecia in numbers which did not in any way approach those produced in Group (1). Four asci were of this type.
(4) Segregation patterns in which two of the eight spores from an ascus yielded protoperithecial cultures and the other six yielded non-protoperithecial cultures. Sixteen asci of this kind were observed.
(5) Segregation patterns in which six of the spores from an ascus yielded protoperithecial cultures and the other two gave non-protoperithecial cultures. Six asci of this type were detected.

If a binocular dissecting microscope were employed to ascertain the presence or the absence of protoperithecia in the isolates, the classification would not correspond to the system just outlined wherein scoring was done with the unaided eye.

Because of the peculiar segregation ratios obtained from the 142 asci, a conclusive analysis of the protoperithecial factor cannot be made. However, certain information was obtained which may be of value in further studies. Some of the segregants obtained were new types which had the combined characteristics of both parents. Thus, pigmented and non-pigmented protoperithecial cultures of both compatibility groups were isolated.

Eighty-four of the 142 asci analyzed showed 1:1 segregation for protoperithecia, and of these 70 contained spores which yielded cultures

like the parental ones, and 14 contained spores some of which yielded cultures unlike the parental ones.

Spermatiophores and spermatia were not taken into account when this cross was made. Both parents produce them and an examination of the segregants for this character indicated that a quantitative segregation for spermatiophores was being obtained. This was particularly noticed in those segregation patterns (84 asci) in which the protoperithecial complex segregated in a 1:1 fashion. Those isolates which formed no protoperithecia generally produced noticeably more spermatiophores and spermatia than those which did.

FRUITING BEHAVIOR

Considerable sterility is often encountered in this fungus, thus making it difficult to obtain mature fruiting bodies and ascospores. Some of the factors which may produce this sterility are: 1) compatibility factor, 2) environmental conditions, and 3) capacity to produce protoperithecia. It became apparent that this third factor was of great importance in determining the sterility or fertility of certain crosses. Furthermore, this factor became more significant when it was observed that, aside from known genetic segregation for protoperithecia, the capacity to produce protoperithecia could be modified by subculturing. When such modification is encountered, it is consistently in the direction protoperithecial → non-protoperithecial and is believed to be the result of a mutation. Concommitant with this change, a loss in pigmentation occurs. Such non-protoperithecial strains are then only fertile in combination with protoperithecial strains of the opposite compatibility.

Genetic segregation for protoperithecia also determined the sterility or fertility of certain crosses. Data accumulated when the F_1 segregants were backcrossed to parents A-3 and a-12 indicate that, excluding other factors, only those matings are fertile in which at least one of the strains has the capacity to produce protoperithecia. The non-protoperithecial segregants were fertile with a-12 but infertile with A-3, in crosses in which both mating types were present. When crossed with *207.1.5*, a protoperithecial isolate, the non-protoperithecial segregants which were infertile with A-3 were then fertile. In certain matings sterility due to ascospore abortion was observed. During the isolation of asci for tetrad analysis, occasional perithecia obtained from cross a-12 × A-3 contained asci in which only four of the spores matured and the other four aborted.

The distribution pattern of perithecia produced in Petri-dish matings

differed with the isolates employed. However, for any two strains the pattern was constant under standard culture conditions. Two general types are recognized: Type I, represented by cross 3 × 234.3.8 in FIG. 2A, is characterized by the formation of perithecia on the one side of the culture. (In this case strain 234.3.8 is the protoperithecial strain and 3 is the non-protoperithecial strain.) Type II, as shown by cross 5 × 234.3.8, is distinguished by the formation of perithecia on both sides of the culture, generally as a narrow line where the two protoperithecium-producing strains meet. As cultures become older the line of perithecia may broaden by the progressive formation of perithecia on both sides of the culture dish.

The potential to produce protoperithecia by the strains employed in such matings was found to be important in the determination of the perithecial distribution pattern. When only one of the isolates involved in a cross produces protoperithecia, a Type I pattern results. Type II pattern is obtained when two protoperithecial isolates are mated. Two non-protoperithecial isolates when mated are sterile and yield no perithecia to form a pattern even though they be of opposite mating types. Environmental factors such as light and temperature also modify the patterns produced.

DISCUSSION

The discovery of spermatia of *Gelasinospora calospora* var. *autosteira* removes any distinction between *G. calospora* var. *autosteira* and *G. adjuncta* Cain (**9**). In all probability Cain's *G. adjuncta* represents a separate isolate of *G. calospora* var. *autosteira*. On the basis of morphological descriptions alone, Moreau and Moreau (**29**) have reduced *G. autosteira* and *G. adjuncta* to synonyms of *G. calospora*. However, until the two heterothallic forms *G. adjuncta* and *G. calospora* var. *autosteira* are critically compared, the taxonomy will still remain somewhat in doubt. If they are the same, as appears to be likely, and are to be treated as a variety of *G. calospora*, then *G. adjuncta* Cain would be reduced to a synonym of *G. calospora* var. *autosteira*. It is of interest to note the distribution of the isolates. *G. calospora* var. *autosteira* was isolated from Spanish moss collected in Natchez, Mississippi (**1**) and *G. adjuncta* from dog dung collected in Germany (**9**). It is possible that other isolates of this strictly heterothallic *Gelasinospora* will be made in the intermediate area, since it seems unlikely that the fungus should be restricted to such widely separated localities.

The sexual structures produced when the isolates of *G. calospora* var. *autosteira* develop to their fullest potential are: 1) protoperithecia with

trichogyne-like outgrowths and 2) spermatia on spermatiophores. The former correspond to the female and the latter to the male components of the mycelium. Hermaphroditic isolates are of either mating type, which indicates that the factors for sex and compatibility are separate entities. Basically, *G. calospora* var. *autosteira* is hermaphroditic. Therefore such unisexual isolates as A-3 are best considered as mutants which arose from a normally hermaphroditic isolate. This point of view is in agreement with that of Whitehouse (**40**), who also believes that such unisexual strains are mutants of normally monoecious fungi. This interpretation seems most likely when one considers that the change from hermaphrodite to unisexual occurred frequently in certain isolates employed in this investigation.

Because the spermatia and trichogyne-like structures resemble those of other ascomycetes, it is very likely that future experiments will reveal that the sexual behavior of this species is similar to that of *Bombardia lunata* (**43, 44, 45**), *Pleurage anserina* (**2, 3, 4, 18, 31, 32**), *Sclerotinia gladioli* (**21**), and *Ascobolus stercorarius* (**8**), all of which exhibit some form of heterothallism and produce both functional trichogynes and spermatia. However, it was also observed that when certain isolates were mated, few if any spermatia were produced and yet abundant mature perithecia developed. In this case it may be that the compatible nuclei were brought into association by some other mechanism than spermatization. It is possible that the trichogyne, finding no spermatium, fused with a hypha of the opposite strain. Sun (**37**) believed the ascogonial coil copulated with a hypha of the opposite strain, although she did not observe trichogynes or spermatia.

It has been repeatedly shown by numerous investigators that the environment may influence the expression of sexuality in fungi, plants, and animals. In the fungi, interest has centered about such pyrenomycetous fungi as *Neurospora, Venturia,* and *Gelasinospora,* which are suitable for environmental as well as genetic studies. In *G. calospora* var. *autosteira* the expression of femaleness (protoperithecia) is influenced by the environmental as well as genetic factors. The formation of protoperithecia and perithecia was inhibited at 37° by some unknown mechanism. Hirsch (**27**) observed a similar temperature effect in *Neurospora crassa* and in addition found that a rather strong correlation existed between melanin production and protoperithecial formation. At higher temperatures, where melanin production was reduced, protoperithecial development was inhibited. It is possible that such a relationship as just noted may also occur in *Gelasinospora.* However, the pigmentation of the isolates grown at different temperatures was not critically

studied. It was noted, nevertheless, that aside from the light effects, most isolates producing abundant functional protoperithecia were pigmented.

In certain ascomycetes the factors responsible for the differentiation of morphologically distinct sex organs or gametes and those determining compatibility may both be segregated during meiosis. So far as has been investigated, it has always been found that these factors are at separate loci and may or may not be linked (**31**). Heterothallic forms *sensu* Dodge (**15**) of this nature may segregate to produce unisexual strains as exemplified by *Bombardia lunata* (**43, 44, 45**), *Hypomyces solani* f. *cucurbitae* (**22, 25, 26**), *Neurospora sitophila* (**5, 17**), and *Chalara quercina* (**7**).

In most of these forms there is still a question whether or not the production of protoperithecia or ascocarp initials is controlled by a single factor pair. The results with *Gelasinospora calospora* var. *autosteira* obtained in this investigation indicate that a more complex segregation is occurring than can be explained by single-factor segregation. This situation finds a parallel in some other heterothallic fungi. For example, Aronescu (**5**) was unable to reach any definite conclusion about protoperithecial segregations in *Neurospora sitophila* because of the unusual ascal segregation patterns obtained through the study of serially isolated ascospores. But using the same strain material Dodge (**17**) reported a simple 1:1 segregation for protoperithecia. Dodge, however, used random single ascospores whereas Aronescu used serially isolated ascospores, which may account for the difference in results. Dodge (**17**) studied other wild-type strains, and could not find the same phenomenon occurring: he states: "The writer, after some study of the wild-type races mentioned above, is not in a position to warrant any conclusions whatever on this matter of production of protoperithecia. Receptive bodies may often consist merely of differentiated special hyphal branches which would be called ascogonia." In *Bombardia lunata* Zickler (**44**) found that for the most part a 1:1 segregation for ascocarp initials occurs, but he indicated that sometimes ascocarp initials are produced by the *lan* (male) strains. His *bulb* strains produced both ascogonia and spermatia. The incomplete segregation noticed by these workers as well as that obtained in this investigation would seem to indicate that the factors controlling sex are extremely complex and one may doubt whether complete segregation will ever be obtained. On the other hand, there are other investigators (**7, 22, 25, 26**) who have reported a complete segregation for sex.

Perhaps the clearest case of segregation for ascocarp initials (female)

and non-ascocarp-initials (male) was obtained by El-Ani (**22**) in his revaluation of the data of Hanson and Snyder (**25**) and Hirsch (**26**) on *Hypomyces solani* f. *cucurbitae*. El-Ani isolated the eight ascospores from each of 42 asci and determined the sex by employing a functional criterion, i.e., mating to suitable tester strains. He found a definite 1:1 segregation (138 isolates female: 138 isolates male) for perithecial initials. If a functional rather than a morphological criterion is used for determining the presence of protoperithecia in *G. calospora* var. *autosteira* it is not improbable that a definite segregation ratio may be obtained as in *Hypomyces*.

Many heterothallic pyrenomycetes exhibit great variability in fruiting when cultured in the laboratory. In addition to compatibility factors such phenomena as barrage (**20**), unilateral nuclear migration (**19, 30**), heterocaryotic vigor (**20, 36**), and special fruiting factors (**20, 24, 32, 33, 34, 35**) have been found to influence the fruiting pattern of any one fungus.

The segregation for protoperithecia with its effect on fertility as well as on the distribution of perithecia in cultures of *Gelasinospora calospora* var. *autosteira* is still another phenomenon which may influence fruiting in the pyrenomycetes. A very similar effect was noted by Dodge (**14**), although he did not state whether segregation for protoperithecia was observed. In investigating the perithecial distribution patterns of homocaryotic cultures of *Neurospora tetrasperma* and *Gelasinospora tetrasperma* Dodge found that, in addition to the culture conditions, the distribution of perithecia depended upon the direction of nuclear migration and that the distribution pattern had a genetic basis, although a complex one, and was probably linked with the compatibility factor. It would seem most likely that such a complex phenomenon as sex reproduction is controlled by a combination of genetic factors, both simple and complex, as well as cultural factors.

SUMMARY AND CONCLUSIONS

A study of the heterothallic Ascomycete *Gelasinospora calospora* var. *autosteira* revealed the presence of both spermatia and protoperithecia with trichogyne-like structures on mycelium derived from single ascospores of both mating types. Accordingly, *G. calospora* var. *autosteira* consists of strains which, generally, are hermaphroditic and self-sterile; perithecia are formed only when strains of opposite mating type are mated. The morphology of spermatia and spermatiophores and protoperithecia with trichogynes is described.

A study of environmental factors which may influence fruiting in *Gelasinospora calospora* var. *autosteira* indicates that temperature, light, and media are important. Growth was obtained at all temperatures ranging from 12 to 37° C. At 37° neither protoperithecia nor perithecia were produced by matings between isolates A-3 and a-12. Best ascospore production occurred at 20°, other conditions being favorable.

Light was found to be important in that few or no perithecia or protoperithecia were produced in cultures incubated in the dark. Pigmentation and aerial mycelium were more pronounced in cultures grown in the dark.

The distribution of perithecia in Petri-dish cultures was strongly modified by culturing in the dark, and only partly modified by temperatures of 28 and 30°.

In culturing *G. calospora* var. *autosteira*, such environmental factors must be taken into account. The most suitable conditions employed are as follows: Difco corn-meal agar, 25 ml per plate, 20° C., with natural or artificial light supplied throughout the culture period. In addition, culturing should be conducted in vessels which permit adequate exchange of gases, for a reduction in number of fruiting bodies was noted in dishes which were sealed to exclude the air.

One hundred and forty-two asci from cross A-3 and a-12 were dissected and their spores were analyzed for mating type, pigmentation, and protoperithecia.

The Mt and G loci were found to be linked and located in the same arm of the chromosome. The Mt locus is 2.4 units away from the centromere, whereas the G locus is 8.8 units from the Mt locus and more remote from the centromere. Segregation for protoperithecia was incomplete.

The factor or factors responsible for the production of protoperithecia segregated in a 1:1 ratio in 84 of the 142 asci analyzed. The protoperithecial factor complex appeared to be linked to Mt and G. Although the segregation of protoperithecia is perhaps too complicated for analysis at this time, account should be taken of the fact that the segregation which does occur contributes to sterility. If isolates are unable to produce protoperithecia they will be sterile in certain combinations regardless of the mating-type factor.

Cultural behavior studies made with *G. calospora* var. *autosteira* indicate that certain definite distribution patterns of perithecia may be observed in Petri-dish matings. These patterns may be classed as follows: 1) perithecia distributed so that they are all restricted to one thallus of the two strains employed in the cross, and 2) perithecia dis-

tributed on both sides, appearing on both thalli of the strains involved in the cross. These distribution patterns are correlated with the capacity of the individual isolates to produce protoperithecia when grown alone, but other factors may also influence such patterns.

ACKNOWLEDGMENTS

The writer wishes to express his sincere thanks to Professor Constantine J. Alexopoulos for his stimulating interest in this problem and helpful criticism and guidance in this work. The author also wishes to thank Dr. G. B. Wilson for his kind guidance and valuable help, particularly in the genetic analysis.

DEPARTMENT OF BIOLOGICAL SCIENCES
UNIVERSITY OF IDAHO
MOSCOW, IDAHO

LITERATURE CITED

1. **Alexopoulos, C. J. and Sun Huang Sung.** 1951. A new species of *Gelasinospora*. Mycologia **42**: 723–734.
2. **Ames, L. M.** 1930. A study of some homothallic and heterothallic Ascomycetes. Mycologia **22**: 318–322.
3. ——. 1942. An hermaphroditic self-sterile but cross-fertile condition in *Pleurage anserina*. Bull. Torrey Bot. Club **59**: 341–345.
4. ——. 1934. Hermaphroditism involving self-sterility and cross-fertility in the Ascomycete *Pleurage anserina*. Mycologia **26**: 392–414.
5. **Aronescu, A.** 1933. Further studies in *Neurospora sitophila*. Mycologia **25**: 43–54.
6. **Backus, M. P.** 1939. The mechanics of conidial fertilization in *Neurospora sitophila*. Bull. Torrey Bot. Club **66**: 63–76.
7. **Barnett, H. L.** 1953. A unisexual male culture of *Chalara quercina*. Mycologia **45**: 450–457.
8. **Bistis, G.** 1956. Studies on the genetics of *Ascobolus stercorarius* (Bull.) Schröt. Bull. Torrey Bot. Club **83**: 35–61.
9. **Cain, R. F.** 1950. Studies of coprophilous Ascomycetes. 1. *Gelasinospora*. Canadian J. Res. C, **28**: 566–576.
10. **Catcheside, D. G.** 1951. The Genetics of Micro-organisms. Pitman and Sons, London. 223 pp.
11. **Dodge, B. O.** 1912. Method of culture and the morphology of the archicarp in certain species of the Ascobolaceae. Bull. Torrey Bot. Club **39**: 139–197.
12. ——. 1927. Nuclear phenomena associated with heterothallism and homothallism in the Ascomycete *Neurospora*. J. Agr. Res. **35**: 289–305.
13. ——. 1932. The non-sexual and sexual function of microconidia of *Neurospora*. Bull. Torrey Bot. Club **59**: 347–360.
14. ——. 1935. The mechanics of sexual reproduction in *Neurospora*. Mycologia **27**: 418–438.

15. ——. 1936. Facultative and obligate heterothallism in Ascomycetes. Mycologia **28**: 399–409.
16. ——. 1936. Spermatia and nuclear migrations in *Pleurage anserina*. Mycologia **28**: 284–291.
17. ——. 1946. Self-sterility in "bisexual" heterocaryons of *Neurospora*. Bull. Torrey Bot. Club **73**: 410–416.
18. **Dowding, E. S.** 1931. The sexuality of the normal, giant, and dwarf spores of *Pleurage anserina* (Ces.) Kuntz. Ann. Bot. **45**: 1–14.
19. —— **and A. Bakerspigel.** 1954. The migrating nucleus. Canad. Journ. Microbiol. **1**: 68–78.
20. —— **and** ——. 1956. Poor fruiters and barrage mutants in *Gelasinospora*. Canad. Journ. Bot. **34**: 231–240.
21. **Drayton, F. L.** 1934. The sexual mechanism of *Sclerotinia gladioli*. Mycologia **26**: 46–72.
22. **El-Ani, A. F.** 1954. The genetics of sex in *Hypomyces solani* f. *cucurbitae*. Amer. Jour. Bot. **41**: 110–113.
23. **Emmerson, R. M.** 1948. Effect of furfural on germination of *Neurospora* spores. Jour. Bact. **55**: 327–330.
24. **Esser, Karl.** 1954. Sur le déterminisme génétique d'un noveau type d'incompatibilitié chez *Podospora*. Comptes Rend. Acad. Sci. **237**: 666–668.
25. **Hansen, H. N. and W. C. Snyder.** 1943. The dual phenomenon and sex in *Hypomyces solani* f. *cucurbitae*. Amer. Jour. Bot. **30**: 419–422.
26. **Hirsch, H. E.** 1949. The cytogenetics of sex in *Hypomyces solani* f. *cucurbitae*. Amer. Jour. Bot. **36**: 113–121.
27. **Hirsch, H. M.** 1954. Environmental factors influencing the differentiation of protoperithecia and their relation to tyrosinase and melanin formation in *Neurospora crassa*. Physiol. Plant. **7**: 72–97.
28. **Lindegren, C. C.** 1933. The genetics of *Neurospora*. III. Pure bred stock and crossing-over in *Neurospora crassa*. Bull. Torrey Bot. Club **60**: 133–154.
29. **Moreau, Claude and Mireille Moreau.** 1951. La genre *Gelasinospora* Dowding. La Mycotheque. Premier Supplément Micromycètes. 39–41.
30. **Olive, L. S.** 1956. Genetics of *Sordaria fimicola*. I. Ascospore color mutants. Amer. Jour. Bot. **43**: 97–106.
31. **Raper, John R.** 1954. Life cycles, sexuality, and sexual mechanisms in the fungi. Sex in microorganisms, pp. 42–81. Amer. Assoc. Adv. Sci., Washington, D. C.
32. **Rizet, G.** 1941. La formation d'asques hybrides dans les confrontations de souches "self-steriles" et de souches "self-fertiles" chez la *Podospora anserina*. Rev. de Myc. **6**: 128–133.
33. ——. 1942. Les resultats d'ordre génétique et le probleme de la sexualité chez l'Ascomycète *Podospora anserina*. Rev. de Myc. **7**: 97–100.
34. ——. 1953. Sur la multiplicité des mécanismes génétiques conduisant des barrages chez *Podospora anserina*. Comptes Rend. Acad. Sci. **237**: 666–668.
35. —— **and Karl Esser.** 1953. Sur des phénomènes d'incompatibilité entre souches d'origine différentes chez *Podospora anserina*. Comptes Rend. Acad. Sci. **237**: 760–761.
36. **Sansome, E. R.** 1946. Heterokaryosis, mating-type factors and sexual reproduction in *Neurospora*. Bull. Torrey Bot. Club **73**: 397–409.

37. **Sun, H. S.** 1953. A comparative morphological and cytological study of *Gelasinospora calospora* (Mouton) Moreau et Moreau, *Gelasinospora autosteira* Alexopoulos and Sun and *Gelasinospora cerealis* Dowding. Ph.D. Thesis, Michigan State College. 56 pp.

38. ——, **C. J. Alexopoulos, and G. B. Wilson.** 1954. A cytotoxonomic study of three species of *Gelasinospora*. Cytologia **19**: 255–264.

39. **Westergaard, M. and H. K. Mitchell.** *Neurospora*. V. A synthetic medium favoring sexual reproduction. Amer. Jour. Bot. **34**: 573–577.

40. **Whitehouse, H. L. K.** 1949. Heterothallism and sex in the fungi. Biol. Rev. **24**: 411–447.

41. **Wilson, G. B. and C. J. Alexopoulos.** 1956. Spontaneous mutation in *Gelasinospora calospora*, a homothallic fungus. Mycologia **48**: 685–689.

42. **Wulker, H.** 1935. Untersuchungen über Tetradenaufspaltung bei *Neurospora sitophila* Shear at Dodge. Ztschr. indukt. Abstamm. u. Vererb.-lehre **69**: 210–248.

43. **Zickler, H.** 1934. Genetische Untersuchungen an einen Heterothallischen Askomyzeten (*Bombardia lunata* nov. spec.). Planta **22**: 573–613.

44. ——. 1937. Die Vererbung des Geschlechts bei dem Askomyzeten *Bombardia lunata* Zckl. Ztschr. indukt. Abstamm. u. Vererb.-lehre **73**: 403–409.

45. ——. 1937. Die spermatienbefruchtung bei *Bombardia lunata*. Ber. deutsch. Bot. Ges. **55**: 114–119.

TAXONOMIC STUDIES IN THE MYXOMYCETES. I. THE TRICHIA FAVOGINEA COMPLEX [1]

MARIE L. FARR

(WITH 18 FIGURES)

The order Trichiales is characterized by light-colored spores and a well-developed capillitium. In the genus *Trichia*, the latter consists of a mass of individual filaments (elaters) which are hollow and externally marked with spiral bands.

The purpose of this study is to clarify the relationships within that group of *Trichia* species characterized by coarsely reticulate spores. This assemblage of species is here called the *Trichia favoginea* complex because *Trichia favoginea* is the oldest named species of the group.

All species of the *T. favoginea* complex except *T. verrucosa* are fairly common and well-distributed throughout the temperate regions (less so in the tropics) of the world. The latter species has been reported from widely scattered areas of all continents, but is apparently rather rare.

The number of species included in this complex has, in the past, varied from one to thirteen, and in most of the recent monographs (Lister, **1894**, **1911**, **1925**; Hagelstein, **1944**; and Martin, **1949**) the four species *T. favoginea* (Batsch) Pers., *T. affinis* de Bary, *T. persimilis* Karst., and *T. verrucosa* Berk. are recognized. These four species, as defined at present, may be distinguished from each other by the following key: [2]

Sporangia distinctly long-stalked. Peridium prominently papillose..*T. verrucosa*
Sporangia sessile. Peridium smooth to delicately punctate-striate
  Elaters 6–8 μ in diameter; reticulations in the form of narrow bands, 2 μ high, smooth or sparsely pitted.....*T. favoginea*

[1] The present paper represents a portion of a Ph.D. dissertation completed at the State University of Iowa under the guidance of Dr. G. W. Martin and Dr. C. J. Alexopoulos. The writer wishes to thank Dr. Alexopoulos for reading the manuscript and extending most helpful criticism before it was submitted for publication.

[2] Martin, **1949**, slightly modified.

Elaters 4–6 μ in diameter; reticulations in the form of pitted bands rarely over 1 μ high.
Reticulation complete or nearly so; bands up to 1 μ high.....*T. affinis*
Reticulation incomplete, sometimes reduced to pitted, wart-like ridges up to 0.5 μ high.....*T. persimilis*

This study is an attempt to evaluate the characters that have been used to separate even these four species on the basis of their distinctness and degree of constancy as shown by examination of specimens of all four, determined by well-known students of the Myxomycetes, including Rostafinski, Karsten, Massee, Farlow, Rex, Wingate, Morgan, A. Lister, G. Lister, Macbride, H. C. Gilbert, Hagelstein, Emoto, and Martin. Specimens were studied from the herbaria of the State University of Iowa (IA), New York Botanical Garden (NY), University of Strasbourg (STR), University of Helsinki (H), and the Royal Botanic Gardens, Kew (K).

MATERIALS AND METHODS

The quantity of material studied and its geographical distribution were as follows: *T. favoginea:* IA, 63 specimens; NY, 6; STR, 3; H, 3; total, 75. North and Central America, Caribbean area, continental Europe, British Isles, Asia, Japan. *T. affinis:* IA, 47; NY, 15; STR, 3; K, 2; total, 67. North, Central, and South America, Caribbean area, continental Europe, British Isles, Asia, Japan, New Zealand, Australia. *T. persimilis:* IA, 52; NY, 6; STR, 6; H, 4; K, 2; total, 69. North America, Caribbean area, continental Europe, British Isles. *T. verrucosa:* IA, 6; NY, 5; K, 1; total, 12. North and South America, Caribbean area, New Zealand, Australia.

Temporary slides for microscopic examination were made in 2–3% KOH, while Gurr's medium was used for permanent mounts. The latter could be directly applied on an aqueous preparation and dried rapidly, but had the disadvantages of bleaching and shrivelling the spores to such an extent that drawings had to be made from temporary mounts. All microscopic drawings were prepared with the aid of a camera lucida, using a Zeiss 15 × ocular and a Zeiss oil immersion objective, 90 ×, N. A. 1.25. They were reduced by one-half in reproduction.

OBSERVATIONS

Since the criteria used to separate the four presently recognized species are 1) presence or absence of distinct stalks, 2) diameter of the

elaters, 3) completeness, height, and pittedness of the episporic markings, and, to a lesser degree, also the shape and appearance of the sporangia, observations were concentrated on the variability of these characters in the various collections available.

STALKS

The fruiting bodies of almost all specimens of *T. favoginea* were sessile, but in one specimen, several sporangia were surrounded by a membrane formed from the hypothallus, and several European collections contained distinctly substipitate or even short-stipitate sporangia;

TABLE I

SUMMARY OF THE MOST PERTINENT DATA ACCUMULATED, TO DEMONSTRATE THE HIGH DEGREE OF INTERGRADATION BETWEEN THE SO-CALLED SPECIES TRICHIA FAVOGINEA AND T. PERSIMILIS

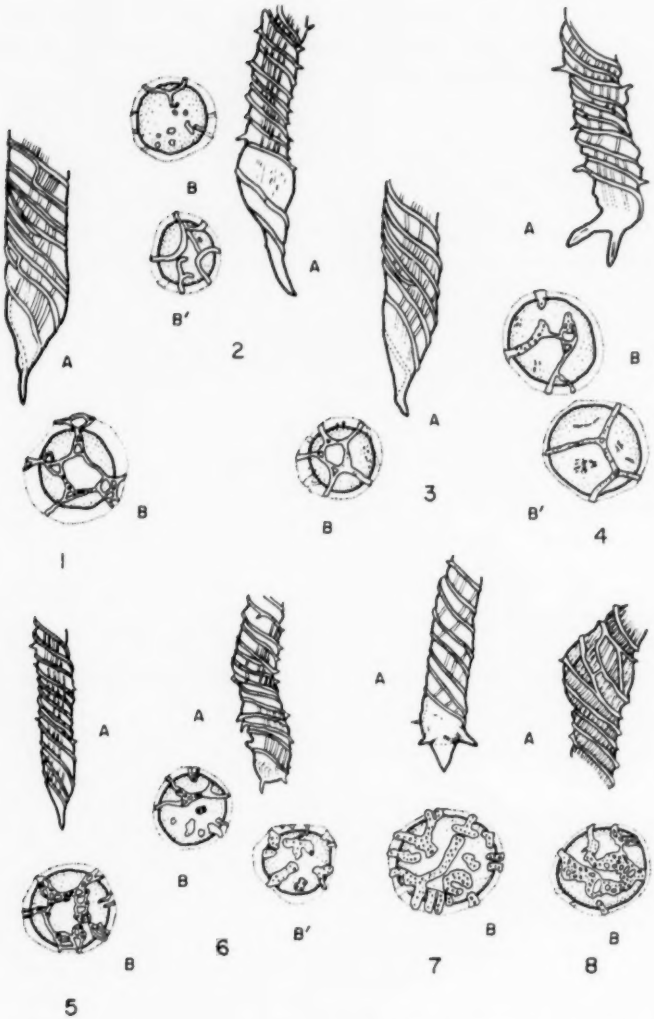
	Approx. % of specimens investigated
Definitely "*Trichia favoginea*" in spores and capillitium	33
Definitely "*T. persimilis*" in spores and capillitium	36
Intermediate: Total	31
"*T. favoginea*" spores, but narrow or border line (6μ) capillitium	14
"*T. persimilis*" spores and wide or borderline capillitium	7
Intermediate or borderline in spores and capillitium	7
Microscopic characters of "*T. favoginea*," sporangial shape of "*T. persimilis*" [a]	8
Microscopic characters of "*T. persimilis*," sporangial shape of "*T. favoginea*" [a]	1

About four gatherings consisted of sporangia showing a complete intergradation of shapes ranging from one extreme to the other.

[a] These figures should be considered minimal, since only well-preserved specimens containing sporangia of determinable shape could be included.

these stipes or stipe-like bases were apparently prolongations of the vein-like deposits of the hypothallus connecting certain sporangia. No suggestion of a stipitate condition was found in material examined of *T. affinis* and *T. persimilis*.

Only a dozen collections of *T. verrucosa* were available for study. In all of these except one gathering from Jamaica, B. W. I., the sporangia were borne on fascicles of fused, thick, rugose, long and often rather weak stalks (FIG. 15, D). The Jamaican collection, because of its sessile habit, was at first referred to *T. favoginea;* but because of its prominently papillose peridium and the general appearance of its episporic network it is believed that this specimen may be a depauperate fruiting of *T. verrucosa*.



FIGS. 1–4. *Trichia favoginea*, × 1258. A. Portion of elater. B. Spore. FIGS. 5–8. *T. persimilis*, × 1258. A. Portion of elater. B. Spore.

ELATERS

The diameter of these filaments in *T. favoginea* material was mostly 6.5–8.5 μ, rarely to 9.5 μ, and, in about 1/7 of the specimens, 6 μ or less. (See TABLE I for percentage figures of the results gleaned from this investigation.)

In *T. persimilis,* the elaters were 4.5–5.5 μ diameter in 75% of the material studied, 6 μ in 17–18%, and slightly wider in 6%. The elaters of *T. affinis* ranged from 3.5–8 μ, and in markings were similar to those of the other three species. Since capillitial and spore structure are not particularly significant diagnostic features in *T. verrucosa,* they need not be discussed here.

SPORES

The episporic markings found in *T. favoginea* specimens most frequently appeared in the form of incomplete to complete reticulations, sometimes complete only one one hemisphere (FIGS. 1–4); warts and fragments of bands were commonly present in the meshes of the network. In a number of gatherings the deposits on the spore wall consisted of mere fragments arranged in a more or less reticulate pattern and forming, at best, an incomplete network on one side (FIG. 2). More often the bands were narrow, but in some fruitings their width as well as their degree of prominence was inconstant. Minute pits were observed in the bands of at least a few spores of each specimen and sometimes consistently. In one specimen, epispores within one sporangium exhibited a range of variation from a complete net formed by medium-wide, pitted bands (as in FIG. 4, B) with scattered or grouped warts in the meshes, to a pattern of irregularly anastomosing, very wide bands with pits (as in FIGS. 6B, 7, 8) or composed of tiny meshes, as characteristic of *T. persimilis.*

Typical spores of *T. persimilis* sporangia were marked with low, wide (c. 1.5 μ), distinctly pitted strips and warts not combined into a network (FIGS. 7, 8). In a considerable number of collections, however, the spore bands branched to form a partial to nearly complete network (FIG. 6, B). At times the above variations all occurred within one specimen (FIG. 6). Furthermore, a portion of the spores of some collections exhibited a reticulation of narrow bands (0.5–1 μ), or of both wide and narrow bands, with large warts present in the meshes. FIG. 5 illustrates that the episporic bands may themselves consist of intricate networks with minute meshes, as depicted by Morgan (**1893**) for this group of species.

The episporic pattern in the material of *T. affinis* varied extensively, ranging from low, broad, indistinct warts and fragments (as characteristic of *T. persimilis*) to a sharp, nearly to entirely complete, uniformly large-meshed network composed of high (usually pitted) ridges, with warts and fragments in the meshes (as in typical *T. favoginea*) (FIGS. 9–14). Varying degrees of incomplete reticulation were found between those extremes.

SPORANGIA

While sporangial shape is not a key character in separating the sessile species, it nevertheless appears to be associated to a certain extent with the character of the sporangial contents, which has largely determined the species concept in this group.

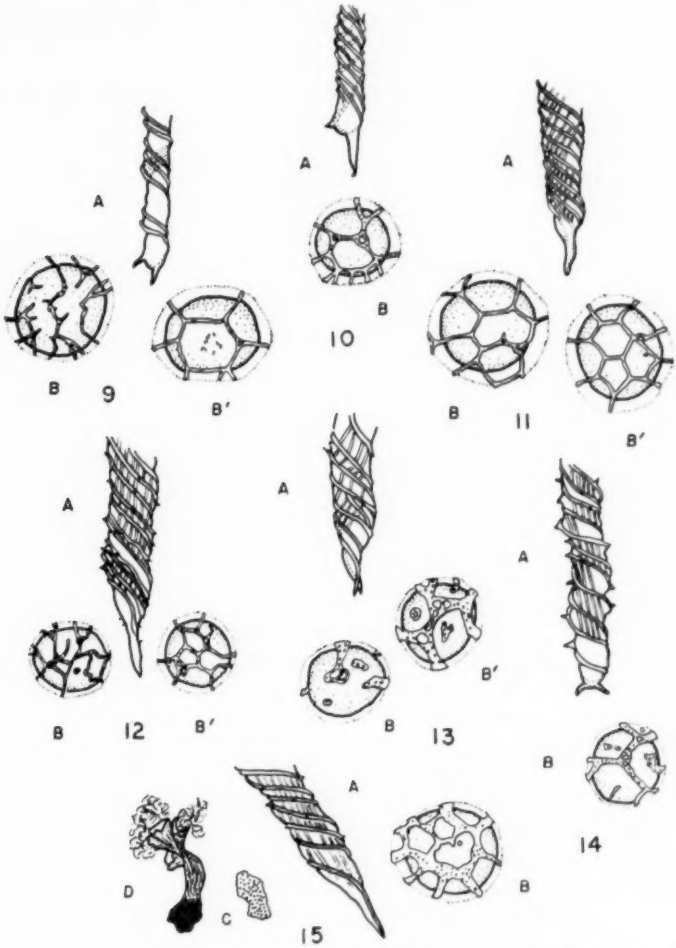
T. favoginea, for example, is characterized in descriptions and illustrations by tall-clavate or more or less cylindrical sporangia, whereas for *T. persimilis,* globose to very broadly clavate sporangia are considered normal. *T. affinis* is generally regarded as an intermediate form, and its sporangia are described as subglobose to obovoid. Hagelstein (**1944**) points out the trend from small, globose sporangia in *T. persimilis* toward larger and more clavate forms in *T. affinis.*

In the present study, sporangia of *T. favoginea* collections tended, on the whole, to be decidedly taller than broad (with occasional exceptions), while those of *T. affinis* and *T. persimilis* were largely more or less equidimensional, sometimes even broader than tall. There seems to be no difference among these species in manner of dehiscence. Recent literature (Macbride, **1899** and **1922**; Macbride and Martin, **1934**; Martin, **1949**) refers to woolly wefts of capillitium above the dehisced sporangia as typical of *T. favoginea.* Macbride and Martin state for the latter species: "a . . . species recognizable at sight, after the peridia break, by the aggregate capillitium constantly in evidence above the abandoned vasiform peridia"; and for *T. persimilis:* "It never shows at maturity the brilliant golden yellow fluff that hangs in masses about the open and empty vases of *T. favoginea.* . . ."

In the material examined, these capillitial masses were lacking in about one-fourth of the specimens of *T. favoginea,* but present in about half the collections of *T. persimilis,* and in a number of the fructifications of *T. affinis.* In all cases, their presence appeared to be associated with robust fruiting bodies matured under optimum conditions.

The presence of occasional stalked or substipitate sporangia in these three species has already been pointed out. In these, however, the short stalks and stalk-like bases were thin and smooth, or membranous, and

were apparently formed for the greater part as raised extensions of the brown thickenings on the hypothallus. The sporangia of *T. verrucosa*, on the other hand, were supported by long, thick, furrowed stalks, usually several fused, and definite in structure and origin.



FIGS. 9–14. *Trichia affinis*, × 1258. A. Portion of elater. B. Spore. FIG. 15. *T. verrucosa*. A. Portion of elater, × 1258. B. Spore, × 1258. C. Portion of peridium, × 1258. D. Habit, × 10.

DISCUSSION

It is evident from the summarized data for the microscopic studies of the three sessile species that there are two extremes in spore and capillitium pattern. One of these consists of capillitium over 6 μ diameter, and spores with high, narrow, distinct, more or less complete reticulation, with pits present or lacking—typical *"T. favoginea."* At the opposite extreme, and representing normal *"T. persimilis,"* are a capillitium less than 6 μ diameter, and spores with low, commonly broad, often indistinct, non-anastomosing fragments and warts, arranged in a more or less reticulate pattern.

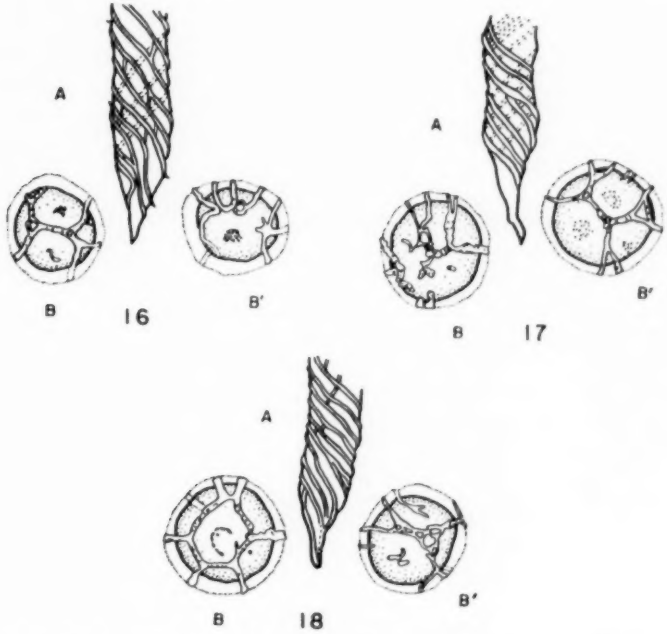
A large number of specimens, on the other hand, are intermediate in their episporic markings, with bands varying in width and height and degree of anastomosis to form a network. The delicately and intricately netted spore bands frequently found in *T. persimilis* (described on page 361) could be interpreted as being a transitional condition between an epispore with wide bands containing large pits, and one with a small-meshed overall reticulation formed by narrow bands.

The capillitium tended to be more stable, the elaters in the greater number of collections being either stout or distinctly slender; TABLE I, however, brings out the fact that in many gatherings, the elaters measured 6 μ and thus were on the borderline. Moreover, among the material studied were several collections with spores of the *T. favoginea* type but capillitium less than 6 μ in diameter, while three or four fructifications contained the type of spores generally found in *T. persimilis* and elaters over 6 μ diameter.

In well-developed fruitings there seems to be a certain amount of correlation between typical microscopic characters of *T. favoginea* with tall-clavate or cylindric sporangia on the one hand, and internal characteristics of *T. persimilis* with rather short or low sporangia of varying shapes, on the other. Though such fruitings certainly manifest enough variation from each other to suggest two distinct species, this study has demonstrated that they should be interpreted as representing two opposite points of development which are linked through a great number of intermediate forms, many of which have been relegated to *"T. affinis."* Each specimen studied of this species either turned out to be such an intermediate form or could be placed without difficulty in the categories of *"T. favoginea"* or *"T. persimilis."* No feature in the form or structure of these specimens was detected that was common to all of them and at the same time set them apart from collections of the other two species. This should justify considering *T. affinis* invalid. The de-

scriptions of *T. favoginea* and *T. persimilis* found in modern literature are obviously based on specimens representing the two extremes in appearance described above.

It might be argued that the elaters alone, being more constant in size, may be used as the diagnostic criterion for separating the two species *T. favoginea* and *T. persimilis*. The facts, however, do not support



FIGS. 16–18. Specimens of *T. favoginea*, *T. affinis*, and *T. persimilis*, respectively, selected to demonstrate the strong similarity among the three "species." A. Portion of elater, × 1258. B. Spore, × 1258.

such an argument. In the first place, there are (as shown in TABLE I) a number of collections that contain elaters more or less 6 μ in diameter (the borderline established at present) and these could not with certainty be placed in either species; thus this measurement seems a rather arbitrary or artificial line of demarcation which does not occur naturally. In the second place it seems likely that the size of the elaters, as well as the degree of development of spines and longitudinal striae, is largely dependent on environmental conditions at the time of sporangial matura-

tion, and their possible interaction with genetic factors. One must not overlook also the possibility that even small differences in concentration of mounting media and in techniques employed by various investigators may result in the procurement of different measurements, due to variable swelling of the structures involved.

That the spores cannot be used to determine species appears quite certain from the data reported and from the illustrations. The sculpture of the spore wall is probably governed by the same factors as already mentioned in the case of the capillitium. The fact that small capillitium is not necessarily associated with imperfectly developed reticulations nor large capillitium with complete netting may indicate that the factors governing such characters are independently inherited or independently influenced by the environment or both.

A strong argument against this line of reasoning would be that the phenotypic combinations resulting in the intermediate forms may be due to hybridization between two distinct species (i.e. *T. favoginea* and *T. persimilis*). At the present time, however, laboratory techniques for growing slime molds have been successfully developed for but few species (none of which belong to *Trichia*), and consequently, controlled experiments to determine or study hybridization and genetics in this group could not be carried out. Until such work is possible, the fruiting phase developed in nature must necessarily serve as the principal tool in taxonomic studies. This is generally in agreement with the procedures followed in the taxonomy of the higher fungi. It seems that any two organisms crossing or fusing so extensively as to produce the large quantity of intermediates cited in this work would be closely enough akin physiologically and genetically to be considered the same species.

CONCLUSIONS

The results of this study indicate that: 1) *T. verrucosa* Berk. appears to be a distinct and valid species, judging from the material examined. The long-stalked, botryoid habit is nearly always characteristic, and in the case of occasional sessile developments, this writer agrees with Hagelstein (**1944**, p. 227) insofar as the diagnostic values of peridium and spores are concerned. 2) Whereas typical *T. favoginea* and typical *T. persimilis* are sufficiently distinct to be readily identifiable, the per cent of fruitings intermediate in, or exhibiting overlapping combinations of, some or all of the diagnostic characters is so high that it is impossible to draw a hard and fast line between these species. 3) All *T. affinis* specimens examined fit somewhere within the range of mor-

phological and structural variations exhibited by *T. favoginea* and *T. persimilis* material.

Until improved techniques make it possible to carry out extensive physiological and genetic experiments with these forms one has no choice, in view of the above, but to consider *T. favoginea, T. persimilis,* and *T. affinis* one and the same species, retaining the oldest name, *T. favoginea,* for this taxon in accordance with the International Code of Botanical Nomenclature (Lanjouw, **1956**), Art. 13 and 57. It is evident from the illustration and brief description by Batsch (**1786**) that his *Lycoperdon favogineum* was based on one of the normally sessile members of this complex. Since these are all here united, this specific epithet—the earliest known in this complex—is valid, even though it cannot be established to which of the three species Batsch's organism may actually have belonged.

On the basis of the above conclusions a new description of *T. favoginea,* incorporating the range of variations represented by the hitherto recognized species, *T. affinis* and *T. persimilis,* is presented below, and the most recent key to species of *Trichia* is adapted accordingly.

TRICHIA FAVOGINEA (Batsch) Pers., emend.

Sporangia sessile (rarely substipitate), usually crowded but occasionally gregarious or solitary (rarely pseudoaethalioid), 0.5–1 mm diam., to 2 mm tall, ranging from irregularly globose, subplasmodiocarpous, or pulvinate to tall-clavate or cylindrical, bright yellow to ochraceous, olivaceous, or yellow-brown, more or less shining or iridescent; dehiscence usually apical, sometimes floriform; peridium membranous, yellow, transparent, nearly smooth or marked with delicate punctate lines; hypothallus membranous, transparent, often with more or less vein-like brown deposits; capillitium yellow, 4–8 μ diam. (rarely more or less), sometimes with swellings, marked with 3–5 smooth to spiny spiral bands usually connected by longitudinal striations; apices pointed, blunt, or rounded and spiny, or furcate; spores yellow to ochraceous or orange in mass, pale to bright yellow by transmitted light, their markings ranging from a pattern of large, irregularly shaped warts or low, broad, pitted bands forming an irregular or fragmentary network, to a more or less complete reticulation of high, narrow, smooth or minutely pitted ridges; plasmodium watery white or white to yellow (fide Martin, **1949**).

?Clathrus turbinatus Huds. Fl. Angl. 632 (1778); Bolt. *t. 94, f. 3.*
Lycoperdon favogineum Batsch, Elench. Fung. Contin. **1**: 257. 1786.
Sphaerocarpus chrysospermus Bull. Hist. Champ. Fr. 131. 1791.

Stemonitis favoginea Gmel. Syst. Nat. **2**: 1470. 1791.
?*Trichia turbinata* With. Bot. Arr. Brit. Pl. *2* ed. **3**: 480. 1792; teste Sow. Engl. Fungi **1**: *pl. 85.* 1797; Berk. in Smith Engl. Fl. **5**: 320. 1836; Cke. Handb. **1**: 405. 1871.
?*T. nitens* Pers. Obs. **1**: *62.* 1796.
?*T. ovata* Pers. Obs. **2**: 35. 1796.
T. chrysosperma Lam. & DC. Fl. Fr. **2**: 250. 1805.
?*Physarum contextum* Spr. Anleitung **2**(1): *20. f. 11–13.* 1817.
Trichia persimilis Karst. Not. Saellsk. Faun. Fl. Fenn. **9**: 353. 1868.
T. affinis de Bary; Fuckel, Jahrb. Nass. Ver. Nat. **23–24**: 336. 1870.
T. Jackii Rost. Monog. *258.* 1875.
T. abrupta Cooke, Ann. Lyc. N. Y. **11**: 404. 1877.
T. proximella Karst. Bidr. Finl. Nat. Folk **31**: 139. 1879.
T. Balfourii Mass. Jour. Roy. Micr. Soc. **1889**: 339. 1889.
T. sulphurea Mass. Jour. Roy. Micr. Soc. **1889**: 339. 1889.
T. intermedia Mass. Jour. Roy. Micr. Soc. **1889**: 341. 1889.
T. Kalbreyeri Mass. Jour. Roy. Micr. Soc. **1889**: 344. 1889.
T. pulchella Rex, Proc. Acad. Phila. **1893**: 366. 1893.

ADAPTATION OF KEY [3]

Subplasmodiocarpous to sporangiate, sessile, rarely short-stalked.
  Elaters with two spiral bands.
  Elaters with three or more spiral bands.
    Spores spinulose to minutely warted.
    Spores more or less reticulate.
      Reticulations delicate.
      Reticulations prominent, coarse.....*T. favoginea*
Sporangiate, stalked.
  Spores reticulate.
    Reticulations prominent, coarse; elaters with short tips.....*T. verrucosa*
    Reticulations delicate, often incomplete; elaters with long-tapering tips.
  Spores warted or spiny.

SUMMARY

A representative number of specimens of *Trichia favoginea* (Batsch) Pers., *T. affinis* de Bary, *T. persimilis* Karst., and *T. verrucosa* Berk. were examined to determine the constancy of the characteristics used to distinguish among these species.

The long-stalked habit and papillose peridium of *T. verrucosa* appear to be sufficiently distinct and constant to justify its continued recognition as a valid species.

[3] Martin, **1949**, p. 49.

Of the total number of collections studied of the other three species, approximately one-third represented typical *T. favoginea;* another third, *T. persimilis;* and the entire remainder consisted of intermediate specimens possessing characteristics of both above species. No structural feature was detected which was common to all specimens of *T. affinis* and at the same time set them apart from material of the other two sessile species.

It is therefore proposed here to unite the three sessile species under the name of *T. favoginea* (Batsch) Pers. in accordance with the International Code of Botanical Nomenclature, and to recognize *T. affinis* de Bary and *T. persimilis* as synonyms thereof.

A new description of *T. favoginea,* incorporating the variations represented by *T. affinis* and *T. persimilis,* is presented.

DEPARTMENT OF BOTANY
STATE UNIVERSITY OF IOWA
IOWA CITY, IOWA

LITERATURE CITED

Batsch, August J. G. C. 1786. Elenchi Fungorum Continuatio **1**: *257, pl. 173a & b.*

Fuckel, K. W. G. L. 1870. Symbolae mycologicae. Jahrb. Nass. Ver. Nat. **23–24**: 336.

Hagelstein, Robert. 1944. The Mycetozoa of North America. 306 pp., *16 pl.* (4 col.). Publ. by the author. Mineola, N. Y.

Lanjouw, J., et al. 1956. International Code of Botanical Nomenclature. International Bureau for Plant Taxonomy and Nomenclature. Utrecht, Netherlands.

Lister, Arthur. 1894. A Monograph of the Mycetozoa. 224 pp., *51 figs., 77 pl.*

——. 1911. A Monograph of the Mycetozoa. ed. *2* (revised by Gulielma Lister). *302* pp., *56 figs., 200 pl.*

——. 1925. A Monograph of the Mycetozoa. ed. 3 (revised by Gulielma Lister). xxxii + *296* pp., *60 figs., 222 pl.* (many col.).

Macbride, Thomas H. 1899. The North American Slime-Moulds. xvii + 231 pp., *18 pl.*

——. 1922. The North American Slime-Moulds. ed. *2.* xvii + *299* pp., *23 pl.*

—— **and G. W. Martin.** 1934. The Myxomycetes. ix + *339* pp., *21 pl.*

Martin, G. W. 1949. Fungi. Myxomycetes. N. Am. Flora **1**(1): 1–151. Bibliography by Harold W. Rickett, pp. 153–173.

Morgan, Andrew P. 1893. The Myxomycetes of the Miami Valley, Ohio. Jour. Cincinnati Soc. Nat. Hist. **16**: 20–21. April, 1893.

Sprengel, Kurt. 1817. Anleitung zur Kenntnis der Gewaechse. II. Theil, 1. Abt., ed. *2.* 502 pp., 10 copper pl. (col.). Halle.

ANTIBIOTICS FORMED BY SPECIES OF EMERICELLOPSIS

F. Kavanagh, D. Tunin and G. Wild

The antibiotic substance cephalosporin N (a penicillin) is formed by two species of *Cephalosporium*. A study of the nutrition of these two fungi revealed that the production of cephalosporin N was stimulated by D-methionine (**4**) in the culture medium. Grosklags and Swift (**3**) showed that one of the fungi, *C. salmosynnematum*, was a new species of *Emericellopsis* van Beyma. The response of the other six species of *Emericellopsis* to methionine was investigated and the antibiotics formed were identified.

Seven species and one presumed member of the genus *Emericellopsis* were tested for production of antibiotics in two different media. Six of the species were obtained from Dr. Myron Backus of the University of Wisconsin. One, *E. salmosynnemata* Grosklags and Swift, was received from Dr. B. H. Olson of the Michigan Department of Health with the designation of *Cephalosporium salmosynnematum* 3590A–UV47. The presumed species was *Cephalosporium* sp. (CMI 41,937; ATCC 11,550) which was the fungus (**1**, **2**) used to make cephalosporins C, N, and P.[1] Stock cultures of the fungi were grown at 30° C on Czapek-Dox agar, on which the appearances of the eight species were quite different.

The ability of these fungi to form cephalosporins N and P was tested in two media used in previous work with *E. salmosynnemata* and *Cephalosporium* sp. (**4**). One medium, corn-meal–soybean-meal, contained corn meal 3.5%, soybean meal 3.5%, ammonium sulfate 0.1%, and calcium carbonate 1% in tap water. The other medium contained dextrin (white potato) 3.5%, carob protein 3.5%, ammonium sulfate 0.1%, calcium carbonate 1% in tap water. The carob-protein, derived from *Ceratonia Siliqua*, was obtained from Morningstar, Nicol, Inc. of New York, N. Y. Quantities of 100 ml of medium were put into 500 ml wide-mouth Erlenmeyer flasks. The mouth of each flask was closed by a pad composed of two milk-filter discs. Incubation of inoculated flasks was at 28° C on a rotary shaker (Gump) for four or

[1] Although it has been shown that cephalosporin P consists of several similar antibiotics, no effort was made to differentiate between members of the group.

five days. Assay for cephalosporin N, a penicillin, was by means of a disc-plate method employing *Salmonella enteritidis* as the test organism. Cephalosporin P was inactive in this test. Identification of the antibiotics was by a paper chromatographic method (**4**). Cephalosporin N has an Rf of 0.2–0.25 and cephalosporin P an Rf of 0.84–0.94. Cephalosporin N was active against *S. enteritidis* as well as *Staphylococcus aureus* whereas cephalosporin P was active against the latter but not the former organism. Thus the two antibiotics could be differentiated by both Rf values and antibacterial activity. The kind of antibiotic

TABLE I

FORMATION OF CEPHALOSPORINS N AND P BY EIGHT SPECIES OF EMERICELLOPSIS

Species of *Emericellopsis*	Antibiotic	Cephalosporin N, mcg/ml			
		Media			
		Carob-protein		Corn-meal	
		DL-methionine		DL-methionine	
		0	0.2%	0	0.2%
humicola	P	<50	<50	<50	<50
minima	N	<50	100	<50	100
mirabilis	N	<50	100	<50	100
salmosynnemata	N	<50	110	120	280
sp. (CMI 41, 937)	N, P	<50	190	90	250
terricola	—	<50	<50	<50	<50
terricola var. *glabra*	N	570	300	220	290
W.S.F. 47	N	60	130	200	150

could be determined by paper chromatography even when the concentration of antibiotic was too low to measure by the plate assay method.

The eight species were grown in the two media with and without added DL-methionine. The growth of all of the species was good with both media. *E. salmosynnemata* and *Cephalosporium* sp. gave considerably increased concentrations of cephalosporin N when a small amount of D-methionine was added to the medium (**4**). Appreciable amounts of cephalosporin N were formed in the two media by only four species, *Cephalosporium* sp. (CMI 41,937), *E. salmosynnemata, E. terricola* var. *glabra,* and W.S.F. 47, of which apparently only the first two responded to DL-methionine. The results are summarized in TABLE I.

The species CMI 41,937 was the only one to form both cephalosporins N and P. Cephalosporin P was the only antibiotic found in

the culture filtrates from *E. humicola*. However, production of antibiotics by it was so poor in the two media used that we would not want to conclude that it formed only cephalosporin P. No antibiotics were detected in the culture filtrates of *E. terricola*. Slight amounts of cephalosporin N were detected in culture filtrates of *E. minima* and *E. mirabilis*. Thus the ability to form cephalosporin N was common to six of the eight species of *Emericellopsis*. Grosklags and Swift reported that all seven of their entities of the genus (they did not report on *Cephalosporium* sp. CMI 41,937) formed an antibiotic which, from its activity against *Salmonella typhimurium* and *S. gallinarum*, could be cephalosporin N. Thus our observations on the same species confirm and extend theirs.

ANTIBIOTICS MANUFACTURING AND DEVELOPMENT DIVISION
ELI LILLY AND COMPANY
INDIANAPOLIS 6, INDIANA

LITERATURE CITED

1. **Abraham, E. P., G. G. F. Newton and C. W. Hale.** 1954. Purification and some properties of cephalosporin N, a new penicillin. Biochem. Jour. **58**: 94–102.
2. **Burton, H. S. and E. P. Abraham.** 1951. Isolation of antibiotics from a species of Cephalosporium; cephalosporins P_1, P_2, P_3, P_4, and P_5. Biochem. Jour. **50**: 168–174.
3. **Grosklags, J. H. and M. E. Swift.** 1957. The perfect stage of an antibiotic-producing Cephalosporium. Mycologia **49**: 305–317.
4. **Kavanagh, F., D. Tunin and G. Wild.** 1958. D-methionine and the biosynthesis of cephalosporin N. Arch. Biochem. Biophysics. In press.

SYNCHYTRIUM FULGENS SCHROETER[1]

JOHN S. KARLING

In 1945 Cook collected a species of *Synchytrium* on *Oenothera laciniata* in Louisiana and identified it as *S. fulgens*. However, in studying this fungus I found that the content of the initial cell grows out to form a sorus of sporangia instead of being transformed directly into a sorus as described by Schroeter (**1873**) and Kusano (**1930a** and **b**) for *S. fulgens*. For this reason I (**1954**) established a new species, *S. brownii*, for the Louisiana fungus. Subsequently, it was found to be so commonly and widely distributed in the U.S.A. that I began to suspect that other collectors besides Cook had mistaken its identity. This led me to an intensive examination of the herbarium specimens bearing the label *S. fulgens* in herbaria in North America, South America, Europe and Japan. This study (**1956**) of stained sections showed that, with the exception of two collections on *Oenothera* (*Meriolix*) *serrulata* in Nebraska and on *O. gregi* var. *pringlei* from Mexico, the initial cell of all specimens functions as a prosorus. These results suggested at once that *S. brownii* and *S. fulgens* might be identical and that Schroeter and Kusano had overlooked the presence of a prosorus and were incorrect in their description of *S. fulgens*. Since the publication of these results I have studied a large number of additional specimens of *S. fulgens* on several other *Oenothera* species from Europe and America with the same results, and after four years of study I feel confident that I have carefully examined most, if not all, existing collections of this species.

Inasmuch as type specimens were not designated at the time Schroeter described *S. fulgens* it is difficult to determine which might be type material. However, there are specimens in the herbarium of the University of Geneva, Switzerland, which Schroeter collected on *Oenothera biennis* in the fall of 1872 and on which he, presumably, based his 1873 diagnosis. Since I have been unable to find any other specimens collected by Schroeter prior to this date I am assuming that the Geneva collection is type material, and am designating it as such. Fixed and stained sections of this material as well as that which Schroeter collected in 1857 (Thumen's Mycoth. univ. 922) show that the initial cell functions as a prosorus. Therefore, I am concluding that *S. fulgens*

[1] This study has been supported by a grant from the National Science Foundation.

was incorrectly diagnosed and that my *S. brownii* is identical with it. I have also studied DeBary's (**1884**) *S. oenotherae* and found that its initial cell functions as a prosorus also and is otherwise similar to Schroeter's species. Accordingly, it and *S. brownii* become synonyms of *S. fulgens*.

On the basis of Schroeter's and Kusano's descriptions, I (**1953**) created a new subgenus, *Exosynchytrium*, for *S. fulgens*, because its reported type of development was different from that of the species of other subgenera. The present study and conclusions, however, indicate that this classification was incorrect and that *S. fulgens* belongs in the subgenus *Microsynchytrium* instead. The removal of *S. fulgens* from *Exosynchytrium* eliminated the necessity of this subgenus, but recently I (**1958**) found that *S. callirrhoeae* exhibits the type of development and life cycle described by Schroeter and Kusano for *S. fulgens*. Accordingly, *Exosynchytrium* is retained for this and similar species which may be discovered in the future.

The species on *Oenothera* (*Meriolix*) *serrulata* and *O. gregi* var. *pringlei* from Nebraska and Mexico, respectively, differ from *S. fulgens* by their smaller sporangia and larger sori which develop directly from the initial cells. Therefore, they are retained provisionally in the subgenus *Exosynchytrium*, pending discovery of the manner in which their resting spores germinate. The former species was collected by J. M. Bates, 8–8–1905, in Franklin Co., Nebraska, and later by M. A. Carleton, 26–4–1914, on the same host at Lubbock, Texas. The collection by Carleton has been studied and compared carefully with that of Bates, and they are identical. An intensive search was made for this fungus at Franklin, Nebraska in June, 1957, but I failed to find it. So far as they are known both of these species fit the original diagnosis of *S. fulgens*, but it does not seem likely that Schroeter collected and studied either of them. At least, there are no specimens of them in any of the numerous herbaria in Europe as far as I have been able to discover.

Inasmuch as there was some uncertainty about *S. fulgens* at the time *Exosynchytrium* was proposed this subgenus as well as *Microsynchytrium* was not diagnosed. However, continued study of *Synchytrium* during the past five years has demonstrated the necessity of these subgenera and they are, accordingly, validated by the following diagnoses:

Microsynchytrium subgen. nov.

Orbis longus, cum prosoris, soris, sporangiis, gametangiis, zoosporis, gametis, et sporis perdurantibus; contentis cellae primae emergentibus in sorum externum transformatis; sporis perdurantibus germinatione post quietem ut prosori agentibus.

Long cycled, including prosori, sori, sporangia, gametangia, zoospores, gametes and resting spores; initial cell functioning as a prosorus, content emerging to form a vesicle which cleaves into a sorus of sporangia or gametangia; resting spore functioning as a prosorus in germination.

Exosynchytrium subgen. nov.

Orbis longus, cum soris, sporangiis, gametangiis, zoosporis, gametis, et sporis perdurantibus; sporangiis et gametangiis delimitatis intra cellam primam; sporis perdurantibus germinatione post quietem ut prosori agentibus.

Long-cycled, including sori, sporangia, gametangia, zoospores, gametes and resting spores; content of initial cell cleaving directly into a sorus of sporangia or gametangia; resting spore functioning as a prosorus in germination.

SUMMARY

A four-year study of most, if not all, collections labeled *S. fulgens* in herbaria in Europe, Japan, and North and South America has shown that the initial cell functions as a prosorus instead of being transformed directly into a sorus of sporangia as originally described by Schroeter and Kusano. Accordingly, it is transferred from the subgenus *Exosynchytrium* to *Microsynchytrium*, and *S. oenotherae* and *S. brownii* are made synonyms of it.

DEPARTMENT OF BIOLOGICAL SCIENCES
PURDUE UNIVERSITY
LAFAYETTE, INDIANA

LITERATURE CITED

Cook, M. T. 1945. Species of *Synchytrium* in Louisiana. I. Description of species found in the vicinity of Baton Rouge. Mycologia **37**: 284–294.

de Bary, A. 1884. Vergleichende Morphologie und Biologie der Pilze, p. 181.

Karling, J. S. 1953. *Micromyces* and *Synchytrium*. Mycologia **45**: 276–287.

——. 1954. *Synchytrium brownii*, a new species with sexual reproduction. Sydowia **8**: 27–30.

——. 1956. *Synchytrium fulgens* in relation to other species on onagraceous hosts. Amer. Jour. Bot. **43**: 61–69.

——. 1958. *Synchytrium callirrhoae* sp. nov. Amer. Jour. Bot. **45**: 327. 1958.

Kusano, S. 1930a. Cytology of *Synchytrium fulgens*. Jour. Coll. Agric. Imp. Univ. Tokyo **10**: 347–388.

——. 1930b. The life history and physiology of *Synchytrium fulgens* Schroet., with special reference to its sexuality. Jap. Jour. Bot. **5**: 35–132.

Schroeter, J. 1873. *Synchytrium* (*Eusynchytrium*) *fulgens* n. sp. *In* Rabenh. europ. exsic. XVII no. 1601–1700. Hedwigia **12**: 139–144.

THE UTILIZATION OF OLIGOSACCHARIDES BY SOME SPECIES OF CERATOCYSTIS [1, 2]

E. M. Wilson [3] and Virgil Greene Lilly

(with 3 figures)

Albritton's (**1953**) summary of the sugars utilized by fungi for growth shows that fewer species utilize lactose, maltose, sucrose, and raffinose than utilize the monosaccharides (glucose, fructose, and galactose) which these oligosaccharides yield on hydrolysis. If fungi hydrolyze oligosaccharides before utilizing the constituent monosaccharides, the nutrient solution would be expected to contain a mixture of sugars during a part of the period of incubation. Mixtures of sugars may support more rapid, or extensive, growth than equivalent amounts of the individual sugars comprising the mixture (Horr, **1936**; Lilly and Barnett, **1953**; Waters *et al.*, **1953**; Johnson and Johan, **1954**; and Lilly and Barnett, **1956**). Certain sugars appear to be utilized only if a second sugar or other source of carbon is also present (Waters *et al.*, **1953**; Sistrom and Machlis, **1955**; and Machlis, **1957**).

Some species synthesize new oligosaccharides when cultured on media which contain disaccharides. An extensive literature on oligosaccharide synthesis by fungi has been published since 1950 when Bacon and Edelman (**1950**) and Blanchard and Albon (**1950**) reported that yeast invertase produced oligosaccharides as well as glucose and fructose during the hydrolysis of sucrose. The following fungi, among others, have been reported to synthesize exocellular oligosaccharides from certain disaccharides: *Aspergillus* and *Rhizopus* species (Aso and Shibasaki, **1953**); *A. oryzae* (Wallenfels, **1951**); *Saccharomyces fragilis* (Roberts and McFarren, **1953**); *Myrothecium verrucaria* (Crook and Stone, **1953**); *Penicillium roqueforti* (Kurasawa *et al.*, **1953**); *P. chrysogenum* (Giri *et al.*, **1953**); and *P. spinulosum* (Bealing and Bacon, **1953**). The ability of certain species to synthesize exocellular poly-

[1] Published with the approval of the Director of the West Virginia Agricultural Experiment Station as Scientific Paper No. 543.

[2] Based on the M.S. thesis of the senior author.

[3] Present address, Department of Plant Pathology, University of California, Berkeley 4, California.

saccharides is well known (Freeman and MacPherson, **1949**; Goyette, **1951**; and White, **1955**).

The purpose of this study was to correlate the dry weight of mycelium produced by a number of species of filamentous fungi when they were grown in media which initially contained lactose, maltose, sucrose, and raffinose with the identity of the sugars present in the media during growth.

MATERIALS AND METHODS

Single-spore isolates of the following species were studied: *Ceratocystis adiposa* (Butl.) C. Moreau; *C. coerulescens* (Münch) Bakshi; *C. fagacearum* (Bretz) Hunt (2 isolates were used, an albino mutant (A) isolated in this laboratory, and a normal (N) dark isolate); *C. fimbriata* Ell. and Halst.; *C. moniliformis* (Hedgc.) C. Moreau; *C. pluriannulata* (Hedgc.) C. Moreau; and *Thielaviopsis basicola* (Berk. and Br.) Ferr.

The liquid basal medium was the same as that used by Lilly and Barnett (**1953**, **1956**) in studies of sugar utilization. It had the following composition: Asparagine monohydrate 2 g; KH_2PO_4, 1 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; thiamine 100 μg; biotin, 5 μg; Fe^{+++} and Zn^{++}, each 0.2 mg; Mn^{++}, 0.1 mg; Ca^{++}, 10 mg; and double distilled water q.s. 1000 ml. The pH of the basal medium was adjusted so that after autoclaving it was between 6.0 and 6.3. The various sugars were added to aliquots of the basal medium in quantities which supplied 10 g of sugar-carbon per liter. In most of the experiments the sugars were autoclaved with the basal medium for 15 minutes at 15 p.s.i.; in some experiments the media were sterilized by filtration.

Standing cultures were used in these experiments. The culture vessels used were 250 ml Pyrex Erlenmeyer flasks; each flask contained 25 ml of medium. The flasks were capped with 50 ml beakers. The media were inoculated with one 2×2 mm bit of mycelium which was cut from Petri-dish cultures of the test fungi growing on glucose-casein-hydrolysate agar medium. The fungi were incubated at $25 \pm 1°$ C in a room which was illuminated 12 hours daily.

A sufficient number of flasks of each medium was inoculated with each test fungus so that serial harvests could be made. The amount of mycelium produced was determined by collecting the mycelium individually from two flasks on a fine-mesh cloth. After the mycelium was washed with distilled water it was dried over night at 100° C and weighed to the nearest milligram. The results reported are the averages

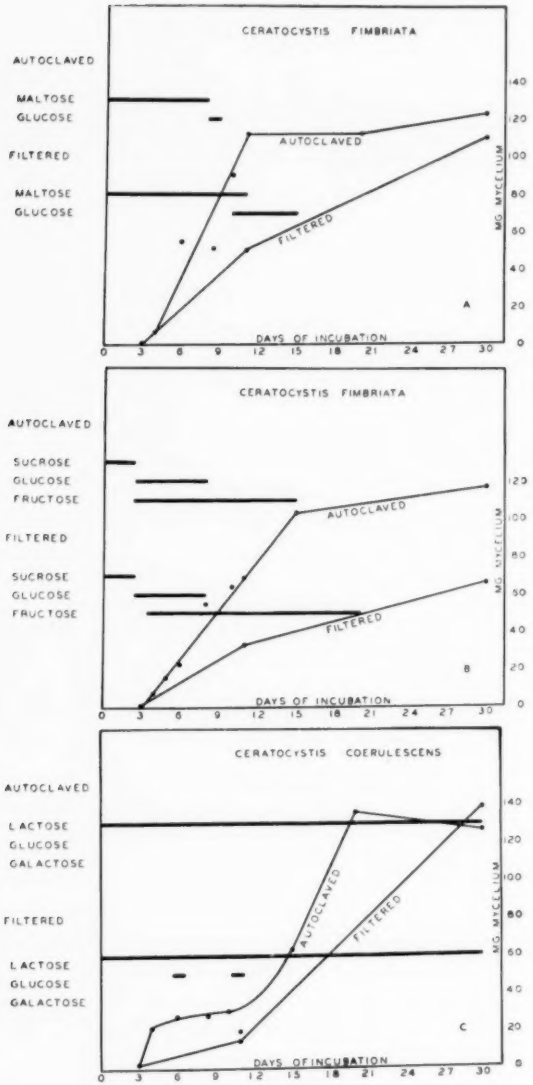


FIG. 1. A–C.

FIG. 1. Growth and residual sugars in media sterilized by autoclaving and filtration. The bars show the periods during which the various sugars were detected by paper chromatography. A. *Ceratocystis fimbriata* on maltose. B. *C. fimbriata* on sucrose. C. *C. coerulescens* on lactose.

of two cultures. The pH values of the media were determined at the time of harvest with a Cambridge pH meter.

The culture filtrates were collected at the time of harvest and the residual sugars determined by descending paper chromatography. Filtrates which were not analyzed immediately were stored at −20° C. The filtrates were not concentrated before analysis. The solvent used in most analyses was the upper phase of a mixture of *n*-butanol-acetic-acid-water (4:1:5, v/v); however, the upper phase of a mixture of *n*-butanol-ethanol-water (4:1:5, v/v) was used in some analyses. This second solvent gave excellent separation of glucose and mannose when used with multiple development. Aniline hydrochloride according to Kurasawa *et al.* (**1952**), and naphthoresorcinol according to Partridge (**1948**) were used in combination to detect sugars. It was found convenient to spray the papers with aniline-hydrochloride reagent first. After the solvent had evaporated, the sheets were sprayed with the naphthoresorcinol reagent. After the second solvent had evaporated the papers were heated at 100° C for 1 to 3 minutes. This method was effective in detecting both aldoses and ketoses as well as oligosaccharides. Known sugars were included in every analysis.

All of the experiments were repeated, some as many as five times.

EXPERIMENTS AND RESULTS

Growth and residual sugars.—Ceratocystis fimbriata, C. coerulescens, and *Thielaviopsis basicola* were cultured for 90 days in media which contained lactose, maltose, sucrose, and raffinose. Twenty-four flasks of each medium were sterilized by autoclaving for each fungus. Harvests were made at 3, 4, 5, 6, 8, 10, 11, 15, 20, 30, 60 and 90 days. Eight flasks of each of the above media for each fungus were sterilized by filtration. This was done because it is known that certain sugars undergo changes on being autoclaved with the other constituents of the medium. Harvests of cultures grown in sterile-filtered media were made at 3, 11, 30 and either 60 or 90 days. However, the residual sugars in both series of media were determined every time the cultures grown in autoclaved media were harvested. Sufficient quantities of sterile-filtered media for analysis were taken with sterile pipettes.

Ceratocystis fimbriata did not utilize lactose or raffinose for growth and these sugars were detected in the media after 90 days of incubation. The results obtained with the maltose media are given in FIG. 1A. No further increase in weight of mycelium occurred in the maltose media after 30 days. Sucrose was utilized by *C. fimbriata;* the essential data are given in FIG. 1B. The weight of mycelium produced in autoclaved

sucrose medium decreased after 30 days, whereas the weight of mycelium in sterile-filtered medium increased to 60 days (96 mg).

Ceratocystis coerulescens grew rapidly in autoclaved maltose (211 mg, 6 days) and sucrose (200 mg, 6 days) media. Growth was slower in sterile-filtered maltose (124 mg, 11 days) and sucrose (82 mg, 11 days) media. Maltose was last detected in autoclaved medium on the eighth and on the fifteenth day in sterile-filtered medium. Glucose was detected in both series of maltose media. Sucrose was last detected in the autoclaved medium on the 4th day, and on the third day in sterile-filtered medium. Glucose and fructose were detected in both series of sucrose media; fructose was found in both media after glucose was no longer detected. The production of oligosaccharides from maltose and sucrose is treated in the next section. *C. coerulescens* grew slowly in lactose media (FIG. 1C). The maximum weight of mycelium produced on lactose was less than that produced on either maltose or sucrose. Both series of media contained lactose after 60 days of incubation. Raffinose was incompletely utilized by *C. coerulescens* since melibiose (two thirds of the raffinose molecule) was present after 90 days of incubation. Fructose was detected only in the sterile-filtered medium, where the rate of growth was slower than in autoclaved medium (FIG. 2A).

Thielaviopsis basicola did not utilize lactose for growth within 90 days. The results with maltose media are presented in FIG. 2B, and with sucrose media in FIG. 2C. The growth of *T. basicola* on raffinose resembled that of *C. coerulescens* (FIG. 2A), except that a lag period of 20 to 30 days occurred before appreciable growth took place. Slightly more mycelium was produced in sterile-filtered (110 mg, 60 days) than in autoclaved (87 mg, 60 days) raffinose medium. Fructose was detected once (10 days) in the sterile-filtered medium. Melibiose was present after 90 days of incubation in both media.

The rate of growth and the dry weight of mycelium produced by *Ceratocystis fimbriata, C. coerulescens,* and *Thielaviopsis basicola* were less when the media were sterilized by filtration than when the media were autoclaved. The utilization of autoclaved sugars was equal to, or better than, sterile-filtered sugars. This was true when either the production of mycelium, or the time required for the original sugar (or its hydrolytic products) to disappear from the medium, was used as a criterion of utilization. One or more of the expected hydrolytic products of the original sugars appeared in the culture filtrates whenever these fungi made appreciable growth. Sucrose was completely hydrolyzed sooner than maltose. Fructose was detected after glucose had disappeared from the media, which suggests that glucose is preferentially utilized in mixtures of these sugars. *C. coerulescens* and *T. basicola*

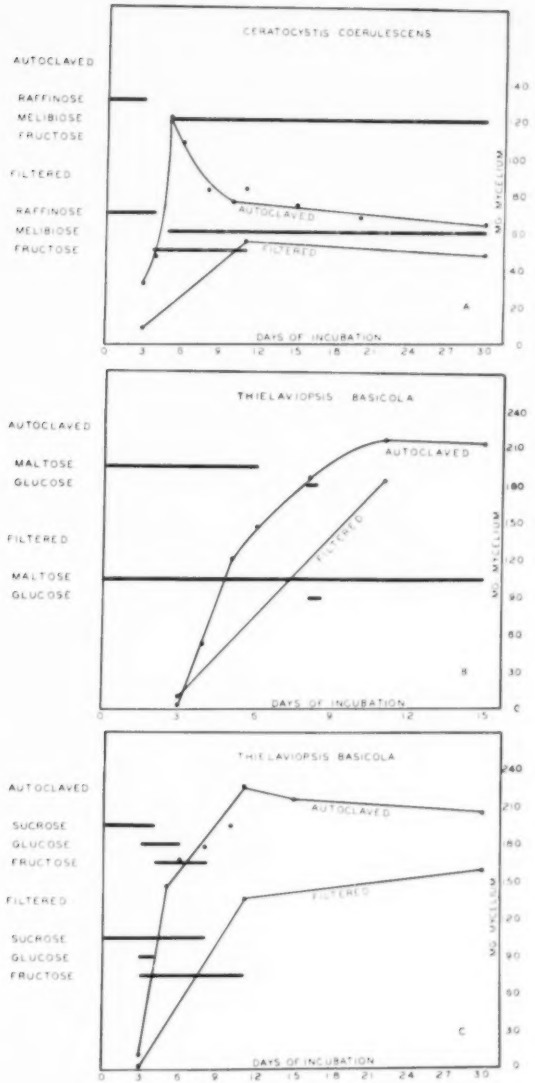


FIG. 2. A–C.

FIG. 2. Growth and residual sugars in media sterilized by autoclaving and filtration. The bars show the periods during which the various sugars were detected by paper chromatography. A. *Ceratocystis coerulescens* on raffinose. B. *Thielaviopsis basicola* on maltose. C. *T. basicola* on sucrose.

made appreciable growth on raffinose. The detection of melibiose in the media at the end of 90 days indicates incomplete utilization. Since fructose was detected only occasionally it is probable that it was utilized almost as rapidly as it was liberated from raffinose.

Synthesis of oligosaccharides.—It was noted in the previous experiments that *Ceratocystis coerulescens* produced two new oligosaccharides when it was grown on the sucrose medium. A further study of the ability of other species to synthesize oligosaccharides was undertaken. The species used included *C. adiposa, C. coerulescens, C. fagacearum* (two isolates), *C. moniliformis,* and *C. pluriannulata.* Samples of the nutrient solutions were taken daily for analysis; the dry weights of mycelium were determined less frequently. Autoclaved media were used in these experiments.

None of the fungi listed above, or *Ceratocystis fimbriata* and *Thielaviopsis basicola* in the previous experiments, were observed to produce new oligosaccharides in media containing lactose or raffinose. Two examples of fungi which produced new oligosaccharides from sucrose are presented in FIG. 3A and 3B. One new oligosaccharide was detected in the culture medium in which *C. fagacearum* (N) had grown for 5 to 7 days. Sucrose was not detected after the sixth day, while some fructose remained in the medium at the end of 20 days incubation. Two new oligosaccharides were detected in the medium in which *C. coerulescens* had grown for 3 days. Sucrose was no longer detected after 4 days, while fructose was present until the eighth day of incubation. The Rg values of the oligosaccharides synthesized by *C. coerulescens* were 0.47 and 0.31. *C. adiposa* and *C. fagacearum* (A and N) produced one new oligosaccharide when they were cultured in the sucrose medium. *C. moniliformis* produced two new oligosaccharides from this medium. The average Rg value of sucrose was 0.69. The Rg values of the new oligosaccharides ranged between 0.30 and 0.50. New oligosaccharides were not observed when *C. fimbriata, C. pluriannulata,* or *Thielaviopsis basicola* was grown in sucrose media. The expected hydrolytic products of sucrose, glucose and fructose, were found in the culture filtrates whether new oligosaccharides were formed or not.

Most of the fungi produced new oligosaccharides when they were grown in maltose medium. The results for two typical fungi are depicted in FIG. 3C and 3D. *Ceratocystis coerulescens* produced one new oligosaccharide from maltose which persisted from the third to sixth day of incubation. The Rg values of these oligosaccharides were about 0.27. The average Rg value of maltose in these determinations was 0.57. Other species which synthesized new oligosaccharides from mal-

tose included *C. adiposa*, *C. fagaccarum* (A), and *C. pluriannulata*. The Rg values of these new oligosaccharides ranged from 0.26 to 0.50. The presence of new oligosaccharides was not detected in the culture filtrates of *C. fagaccarum* (N), *C. fimbriata*, or *Thielaviopsis basicola*.

The chemical nature of the oligosaccharide produced by *Ceratocystis adiposa* from sucrose was investigated further. The new oligosaccharide was separated from the other sugars present in two-day-old filtrate by chromatography, using a large sheet of paper. The area containing the oligosaccharide was cut out and eluted with hot water. The oligosaccharide solution was concentrated and an aliquot of it was hydrolyzed with dilute hydrochloric acid by heating for 90 minutes on a steam bath. The hydrolyzed and unhydrolyzed material was subjected to chromatography. The unhydrolyzed material contained a single slow-moving reducing substance which gave a brown spot with the aniline reagent and a blue to grey spot with an urea-phosphoric-acid reagent (Wise *et al.*, **1955**). The hydrolyzed material no longer contained the slow moving reducing substance present in the unhydrolyzed material. The hydrolyzed material contained a reducing substance which migrated at the same rate as glucose and which gave a brown spot with the aniline reagent. The test for fructose was indistinct with this reagent. However, the presence of fructose in the hydrolysate was confirmed by the use of the urea reagent. It was concluded that the new reducing substance produced by *C. adiposa* from sucrose was an oligosaccharide which contained glucose and fructose units. This is the expected composition of an oligosaccharide formed from sucrose by the pathway proposed by Wallenfels *et al.* (**1953**). The chemical nature of the other oligosaccharides produced by the test fungi was not investigated in detail.

In these experiments, the new oligosaccharides were detected soon after the fungi began to grow appreciably. In all instances, these new oligosaccharides disappeared from the medium before the maximum dry weight of mycelium was produced. It is probable that they were hydrolyzed and the constituent monosaccharides were then utilized.

Synthesis of polysaccharides.—Preliminary experiments showed that *Ceratocystis fagaccarum* (A and N) synthesized polysaccharide-like materials during growth in media containing any one of the following sugars: glucose, sucrose, lactose, maltose, or raffinose. These materials were precipitated from the culture filtrates by the addition of acetone. Samples of the polysaccharide-like materials produced from the above sugars were hydrolyzed by autoclaving 2 hours with 2 *N* sulfuric acid. Chromatographic analysis of these hydrolysates showed the presence of a substance which migrated at the same rate and which gave the same

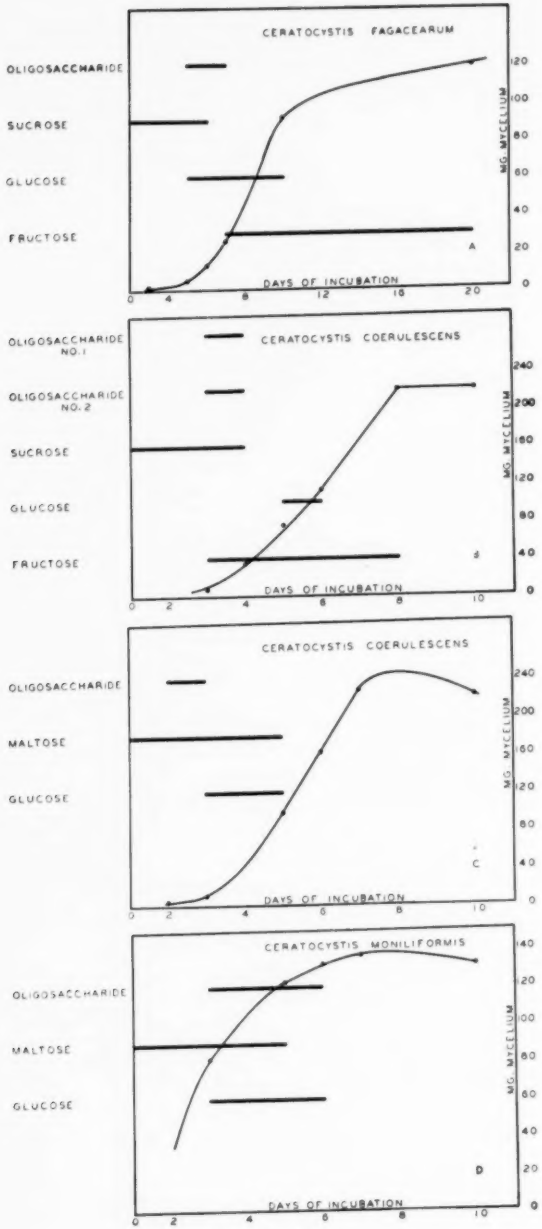

CERATOCYSTIS FAGACEARUM
OLIGOSACCHARIDE
SUCROSE
GLUCOSE
FRUCTOSE
A
DAYS OF INCUBATION
MG. MYCELIUM
CERATOCYSTIS COERULESCENS
OLIGOSACCHARIDE NO. 1
OLIGOSACCHARIDE NO. 2
SUCROSE
GLUCOSE
FRUCTOSE
B
DAYS OF INCUBATION
MG. MYCELIUM
CERATOCYSTIS COERULESCENS
OLIGOSACCHARIDE
MALTOSE
GLUCOSE
C
DAYS OF INCUBATION
MG. MYCELIUM
CERATOCYSTIS MONILIFORMIS
OLIGOSACCHARIDE
MALTOSE
GLUCOSE
D
DAYS OF INCUBATION
MG. MYCELIUM

FIG. 3. A–D.

color reactions as glucose. The hydrolysates of the polysaccharide-like materials from glucose and sucrose media contained a second reducing substance which appeared to be mannose.

In another experiment 20 liters of sucrose-asparagine medium was divided among 40 Fernback flasks, autoclaved, and inoculated with *Ceratocystis fagacearum* (N). After 32 days, the culture filtrate was concentrated under vacuum to 3 liters, and 3 volumes of acetone were added. A black fibrous substance (polysaccharide No. 1) precipitated at once. A tan precipitate formed after the acetone solution stood over night. This material was collected, dissolved in water, and the solution dialyzed over night in cellophane against flowing tap water. The material which remained in the cellophane tube was dried (polysaccharide No. 2). The yield of polysaccharide No. 1 was 1.21 g and that of polysaccharide No. 2 was 0.73 g.

Samples of the two polysaccharide-like materials were autoclaved 2 hours with 2 *N* sulfuric acid. Most of the sulfuric acid was removed with barium carbonate. The filtrates and washings were treated with Amberlite MB-3 to remove ionic materials. The hydrolysates were concentrated and chromatographed. Multiple development with the upper phase of a *n*-butanol-ethanol-water (4:1:5 v/v) mixture resulted in good separation of the reducing substances present in the hydrolysates. Glucose and mannose were found in the hydrolysate of polysaccharide No. 1, while the hydrolysate of polysaccharide No. 2 contained xylose in addition to glucose and mannose.

DISCUSSION

The experimental evidence obtained in this study indicates that lactose, maltose, sucrose, and raffinose are hydrolyzed by the fungi which utilized these sugars for growth. Some of the sugars, especially sucrose, appeared to be completely hydrolyzed before much mycelium was produced (see FIGS. 1B, 2C, 3A, and 3B). The complete hydrolysis of maltose, in general, occurred later in the incubation period than was the case with sucrose (see FIGS. 1A, 2B, 3C, and 3D). Satisfactory evidence of the partial hydrolysis of raffinose by *Ceratocystis coerulescens* and *Thielaviopsis basicola* was obtained. Fructose was detected in the culture medium during the early period of growth, while melibiose was still present in the medium after 90 days of incubation. Similar results

FIG. 3. Growth and oligosaccharide formation. The bars show the periods during which the various sugars were detected by paper chromatography. A. *Ceratocystis fagacearum* (N) on sucrose. B. *C. coerulescens* on sucrose. C. *C. coerulescens* on maltose. D. *C. moniliformis* on maltose.

have been reported by Govindarajan (**1953**) with yeasts. Lilly and Barnett (**1953**) found that *T. basicola* did not utilize melibiose within 18 days when it was the sole sugar added to the medium. The low yield of mycelium obtained when these species were cultured in raffinose medium suggests that fructose was the chief source of carbon utilized (the raffinose molecule is one third fructose). *C. coerulescens* grew slowly on lactose, and glucose was detected only twice in the culture filtrates. This suggests, but does not prove, that the hydrolytic products of lactose were utilized almost as rapidly as they were formed. In the experiments where the original sugar was completely hydrolyzed before much mycelium was produced it is evident that most of the mycelium was produced at the expense of the hydrolytic products. The available evidence does not exclude the possibility that a portion of the original oligosaccharides was utilized by other pathways.

In most instances, growth was more rapid and greater dry weights of mycelium were produced in autoclaved than in sterile-filtered media. The sugars were utilized more rapidly in the autoclaved media. The reasons for this are not known. Other investigators have noted that autoclaving monosaccharides with the basal medium may either increase or decrease growth. Cantino (**1951**) reported a species of *Pythiogeton* to grow more rapily on a fructose-yeast-extract medium when these constituents were autoclaved together than when they were autoclaved separately. Barnett *et al.* (**1953**) found *Thielaviopsis basicola* to make less growth on fructose and glucose when these sugars were autoclaved with the basal medium than when they were sterilized by filtration. Bretzloff (**1954**) obtained considerably more growth of *Sordaria fimicola* when he autoclaved a sucrose medium having a pH value of 4.2 than when sterile-filtered sucrose was added to the basal medium. Sucrose was reported to be about 25 percent hydrolyzed when autoclaved under these conditions (pH 4.2). Waters *et al.* (**1953**) studied another isolate of *S. fimicola* and found it to be unable to utilize sucrose unless another source of sugar-carbon (suitably glucose or fructose) was present. This isolate of *S. fimicola* was used as a biological indicator of possible sucrose hydrolysis under our conditions (initial pH 6.0 to 6.3). Since no more mycelium was produced in experiments which extended to 220 days than could be accounted for on the basis of utilization of asparagine-carbon (Lilly and Barnett, **1956**), it was concluded that the better growth of the fungi used in this work on autoclaved sucrose medium could not be attributed to partial hydrolysis of sucrose.

The production of polysaccharide-like materials by *Ceratocystis fagacearum* was reported by Hoffman (**1953**) and White (**1955**). Neither

investigator identified the constituent sugars in these materials. The presence of glucose and mannose in one polysaccharide and glucose, mannose and xylose in the other suggests that these materials are either heteropolysaccharides, or mixtures of homopolysaccharides.

ACKNOWLEDGMENTS

The authors wish to thank Dr. Ross Davidson for supplying certain cultures used in this work and Mrs. Ruthann Folmer for the final chromatographic study of the polysaccharides produced by *C. fagacearum*. We are indebted to H. L. Barnett for many suggestions and encouragement.

SUMMARY

Ceratocystis fimbriata, C. coerulescens, and *Thielaviopsis basicola* were cultured from 60 to 90 days in media which contained either lactose, maltose, sucrose, or raffinose. *C. fimbriata* did not utilize lactose or raffinose, and *T. basicola* did not utilize lactose. The identity of the sugars present in the media during incubation was determined by paper chromatography. The original sugars were present in the media at the end of the experiments when the sugars were not utilized for growth. The expected hydrolytic products of the above sugars were found when the fungi grew well. Raffinose appeared to be hydrolyzed to fructose and melibiose by *C. coerulescens* and *T. basicola;* melibiose remained in the medium up to 90 days. The following species of the genus *Ceratocysts* synthesized oligosaccharides from maltose or sucrose, or from both of these sugars: *C. adiposa, C. coerulescens, C. fagacearum* (2 isolates), *C. moniliformis;* but *C. pluriannulata, C. fimbriata,* and *T. basicola* did not synthesize detectable amounts of oligosaccharides from either maltose or sucrose. Oligosaccharides were not synthesized in detectable amounts when any of the test fungi were grown in lactose and raffinose media. Sucrose appeared to be completely hydrolyzed before much growth took place. *C. fagacearum* (N) produced 2 polysaccharide-like materials when it was cultured on a sucrose-asparagine medium. Glucose and mannose were detected in the hydrolysate of one polysaccharide, while xylose, glucose and mannose were found in the hydrolysate of the other polysaccharide.

DEPARTMENT OF PLANT PATHOLOGY, BACTERIOLOGY, AND ENTOMOLOGY
WEST VIRGINIA UNIVERSITY
MORGANTOWN, WEST VIRGINIA

LITERATURE CITED

Albritton, E. C. (editor). 1953. Standard values in nutrition and metabolism. WADC Tech. Report 52-301. Table 24. Wright-Patterson Air Force Base, Ohio.

Aso, K., and K. Shibasaki. 1953. Studies on the unfermentable sugars (I–III). Tohoku Jour. Agr. Res. **3**: 337–357.

Bacon, J. S. D., and J. Edelman. 1950. The action of invertase preparations. Arch. Biochem. **28**: 467–468.

Barnett, H. L., V. G. Lilly, and Betsy M. Waters. 1953. The effects of temperature and method of sugar sterilization on growth of *Thielaviopsis basicola*. Proc. W. Va. Acad. Sci. **25**: 27–28.

Bealing, F. J., and J. S. D. Bacon. 1953. The action of mould enzymes on sucrose. Biochem. Jour. **53**: 277–285.

Blanchard, P. H., and N. Albon. 1950. The inversion of sucrose: a complication. Arch. Biochem. **29**: 220–222.

Bretzloff, C. W., Jr. The growth and fruiting of *Sordaria fimicola*. Amer. Jour. Bot. **41**: 58–67.

Cantino, E. C. 1951. Evidence for an accessory factor involved in fructose utilization by the aquatic fungus, *Pythiogeton*. Amer. Jour. Bot. **38**: 579–585.

Crook, E. M., and B. A. Stone. 1953. Formation of oligosaccharides during the enzyme hydrolysis of *beta*-glucosides. Biochem. Jour. **55**: XXV.

Freeman, G. G., and Catherine S. MacPherson. 1949. Studies on metabolic products of the *Penicillium luteum* series. Biochem. Jour. **45**: 179–189.

Giri, K. V., P. L. N. Rao, K. Saroja, and R. Venkataraman. 1953. Enzymic synthesis of oligosaccharides by *Penicillium chrysogenum* Q 176. Naturwissenschaften **40**: 484–485.

Govindarajan, V. S. 1953. Papyrography in the study of raffinose-melibiose fermentation by yeasts. Jour. Sci. and Ind. Res. (India) **12B**: 48–51.

Goyette, L. E. 1951. Studies on the extracellular starch production by species of *Torulopsis*. Kans. Acad. Sci. **54**: 28–35.

Hoffman, P. F., Jr. 1953. Physiology of *Chalara quercina* H. and chemicals for control of oak wilt. Iowa State Jour. Sci. **27**: 187–188.

Horr, W. H. 1936. Utilization of galactose by *Aspergillus niger* and *Penicillium glaucum*. Plant Physiol. **11**: 81–99.

Johnson, S. P., and H. E. Joham. 1954. Some physiological notes on *Sclerotium rolfsii*. 1. Utilization of certain carbon compounds. Plant Dis. Rep. **38**: 602–606.

Kurasawa, H., S. Saito, and N. Honma. 1952. Studies on the metabolism of carbohydrates. Part 3. A study on the paper chromatography of sugar. On the spraying reagents. Bull. Fac. Agr., Niigata Univ. **2**: 47–52.

——, ——, —— **and Y. Yamamoto.** 1953. Production of non-reducing oligosaccharides from sucrose by fungi and the action of the extract from fungal mycelium, and the chemical properties of non-reducing oligosaccharides. Bull. Fac. Agr. Niigata Univ. **4**: 51–61.

Lilly, V. G., and H. L. Barnett. 1953. The utilization of sugars by fungi. W. Va. Univ. Agr. Exp. Sta. Bull. 362T.

—— **and** ——. 1956. The utilization of D- and L-arabinose by fungi. Amer. Jour. Bot. **43**: 709–714.

Machlis, L. 1957. Effect of certain organic acids on the utilization of mannose and fructose by the filamentous water-mold, *Allomyces macrogynus*. Jour. Bacteriol. **73**: 627–631.

Partridge, S. M. 1948. Filter paper partition chromatography of sugars. 1. General description and application to qualitative analysis of sugars in apple juice, egg white and foetal blood of sheep. Biochem. Jour. **42**: 238–248.

Roberts, H. R., and E. F. McFarren. 1953. The chromatographic observation of oligosaccharides formed during the lactase hydrolysis of lactose. Jour. Dairy Sci. **36**: 620–632.

Sistrom, Dorothy E., and L. Machlis. 1955. The effect of D-glucose on the utilization of D-fructose by a filamentous fungus. Jour. Bacteriol. **70**: 50–55.

Wallenfels, K. 1951. Enzymatische Synthese von Oligosaccharide aus Disaccharide. Naturwissenschaften **38**: 306–307.

——, **E. Bernt, and G. Limberg.** 1953. Über den Verlauf der enzymatischen Spaltung von Lactose mit *beta*-Galaktosidase von Schimmelpilzen, *Helix pomatia*, *Escherichia coli* und Kalberdarm. Justus Liebigs Ann. Chem. **584**: 63–85.

Waters, Betsy M., V. G. Lilly, and H. L. Barnett. 1953. The influence of galactose and other sugars on the utilization of sucrose by *Sordaria fimicola*. Proc. W. Va. Acad. Sci. **25**: 23–26.

White, Irene G. 1955. Toxin production by the oak wilt fungus, *Endoconidiophora fagacearum*. Amer. Jour. Bot. **42**: 759–764.

Wise, C. S., R. J. Dimler, H. A. Davis, and C. R. Rist. 1955. Determination of easily hydrolyzable fructose units in dextran preparations. Anal. Chem. **27**: 33–36.

STUDIES OF A UNIQUE PIGMENT COMPLEX AND A PHOTOBIOLOGICAL REACTION IN PENICILLIUM HERQUEI

J. M. Riedhart [1] and C. L. Porter

(with 5 figures)

The significance of pigmentation in the classification of *Penicillium* has long been realized (**28**). Along with other morphological characteristics, species are designated on the basis of conidial color, mycelial color, reverse color, and the presence or absence of diffusible pigments in the substratum (**22, 28**).

Recently a great deal of attention has been devoted to the chemical characterization of these pigments; however, the task is rendered difficult because of their heterogeneous nature. The majority of pigments isolated from *Penicillium* fall into the quinone series, being derivatives of the benzoquinones, napthoquinones and anthraquinones. Other coloring agents in *Penicillium* are classed as antibiotics, aromatic acids, proteins, vitamins and hormones. Informative summaries concerning pigmentation in *Penicillium* and the coloring agents in other molds can be found in recent reviews by Bracken (**5**), Smith (**22**), Raistrick (**17**) and Birkinshaw (**3**).

For years pigments have generally been regarded as end products or by-products serving no useful function in the organism. Recently, however, attempts have been made to elucidate the role they play in fungus metabolism. Raistrick (**17**) suggests that many of the quinone pigments function in oxidation-reduction reactions. This supposition is based upon the fact that in the organism the quinones are generally accompanied by the corresponding quinols (**3**). Smith (**22**) suggests that since a number of these pigments are both antibacterial and antifungal they may have been elaborated as a defense mechanism. It is assumed that the functions of the pigmented vitamins, proteins and hormones are comparable to their roles in higher organisms.

In 1955 the observation was made in this laboratory that upon exposure to sunlight a yellow mycelial mutant, isolated from a strain of

[1] Present address: Cia. Bananera de Costa Rica, Coto Research Station, Golfito, Costa Rica.

P. herquei, suddenly became green (**19, 20**). Because of the unexplored nature of the reaction it was determined that a broad approach, studying many diverse aspects, would be more informative than a quantitative one.

DESCRIPTIONS OF THE ORGANISMS

The following descriptions, as well as all experimental observations, are based upon organisms grown on Czapek's-solution agar as recommended by Raper and Thom (**18**).

The original strain (designated as *P. herquei* Strain I) was isolated from a soil sample collected near Buffalo, New York. It was tentatively identified as *Penicillium herquei* Bainier and Sartory (**18**). The identification was confirmed by Dr. K. B. Raper [2] and by Dr. C. W. Hesseltine.[2]

To date three variants have been obtained from the original strain. The first variant (designated as *P. herquei* Strain II) was isolated from a sector occurring near the edge of a colony of Strain I. It is characterized by its less abundant sporulation, its predominant yellow mycelial color, and its buff colored conidia. In some respects it resembles *Penicillium aureum* Corda (**18**).

The second variant (designated as *P. herquei* Strain III) was isolated from a sector occurring in a colony of Strain II. In all morphological characteristics it appears identical to *P. herquei* Strain II with the exception of its dark colored conidia. This strain was used for all experimental work.

Recently a third variant (designated as *P. herquei* Strain IV) has been isolated from Strain I. The hyphae of this strain are tubercular and the penicilli very irregular, giving the colony a rough appearance. This variant in some characteristics parallels *Penicillium elegans* Sopp (**18**).

It is interesting to note that a few of the morphological characteristics exhibited by these strains, with the exception of color, are also exhibited by the famous "Wisconsin Series" of *Penicillium chrysogenum* (**24**).

EXPERIMENTS IN VIVO

INFLUENCE OF LIGHT: As previously mentioned, the observation was made that the yellow mycelial variant (as well as the others) when exposed to sunlight suddenly became green. It was noted that the color change took place in the mycelium rather than in the medium or in the spores of the organism.

[2] Personal communication.

To determine the length of exposure necessary for the reaction to occur in bright sunlight, colonies were exposed for 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20 minutes. It was found that a period of 4 minutes was necessary to bring about the first trace of green color. As exposure was increased the intensity of the green color increased. It appeared to reach a maximum after an exposure of about 16 minutes. Radiation from fluorescent and mercury vapor lamps also brought about the color change.

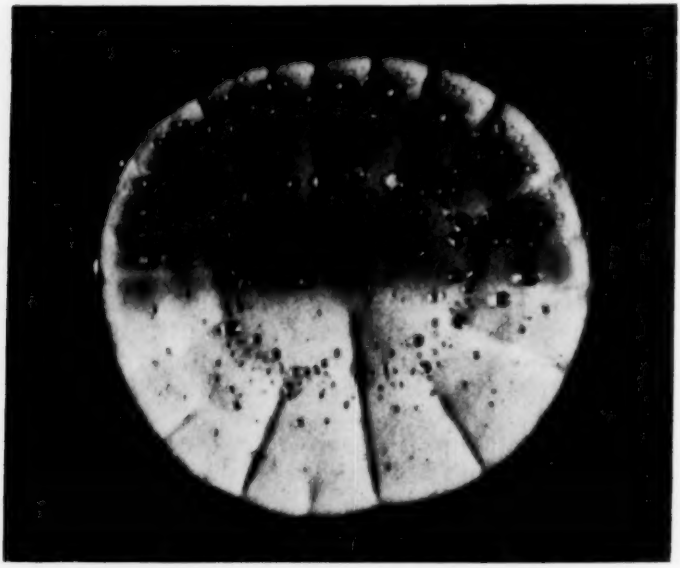


FIG. 1. Colony after exposure to sunlight while one-half (lower half in picture) was covered with black paper.

INFLUENCE OF TEMPERATURE: To rule out the influence of heat in the above reactions the temperature was carefully controlled by the use of water filters. No perceptible difference in temperature was noted between the plates exposed to light and the controls. Other evidence ruling out the influence of temperature was obtained from the following experiments. Plates were exposed to sunlight with one half of the colonies covered with black photographic paper. It was assumed that the black paper would absorb the infra-red rays and filter out the visible and ultraviolet rays. Thereby, the halves of the colonies covered with

black paper would if anything, show an increase in temperature. FIG. 1 shows the results of this experiment. The halves of the colonies covered with black paper remained yellow, while the halves exposed to sunlight became green.

In other experiments the temperature was increased physically by placing plates on a warm plate and increasing the temperature to 100° C or by autoclaving colonies for 15 minutes at 15 lbs steam pressure. Except for a slight browning the colonies remained yellow. Furthermore, the colonies when exposed to light after this treatment became green.

LIVING VS. NON-LIVING ORGANISMS: Since the heated colonies in the above experiment changed color upon exposure to sunlight the question arose as to whether living or non-living organisms were required. Colonies were killed by autoclaving for 15 minutes at 15 lbs steam pressure, and by exposure to the vapors of 37% formaldehyde for 20 minutes. One half of each colony was then exposed to sunlight while the other half was covered with black photographic paper. It was found that non-living as well as living organisms would change color upon exposure to sunlight. In certain cases it appeared that the color change occurred more rapidly in non-living organisms.

TABLE I

INFLUENCE OF AIR, NITROGEN AND CARBON DIOXIDE ON COLOR CHANGE

Treatment	Time for color change
Air	7 min
Nitrogen	28 min
Carbon dioxide	18 min
Pyrogallic acid plus sodium hydroxide	18 min

OXYGEN REQUIREMENT: Colonies were grown on Czapek's-solution agar slants in colorimeter tubes. The air was removed by purging the tubes with nitrogen or carbon dioxide. This process was repeated after 4 hours; then the tubes were sealed and exposed to sunlight. For more specific evidence whether oxygen was essential for the reaction the oxygen was selectively removed from the tubes by means of reacting pyrogallic acid with sodium hydroxide. The results are presented in TABLE I. The fact that all organisms, even those in an apparently oxygen-free atmosphere, eventually changed color can be accounted for by the presence of residual oxygen in the mycelium. Further indications that oxygen was required for the color change to take place are presented in a later section.

INFLUENCE OF LIGHT ON GROWTH: Since the reaction appeared to be a photochemical oxidation, it was deemed essential to determine its influence upon growth. Preliminary experiments were conducted to determine a reliable method of measuring growth. Dry weights, increase in diameter, and cube roots were tried. A measure of the increase in the diameters of the colonies per day proved convenient. All experiments were conducted in a controlled-climate room or in a humidity cabinet. The temperature was kept at $25 \pm 1°$ C. Fluorescent

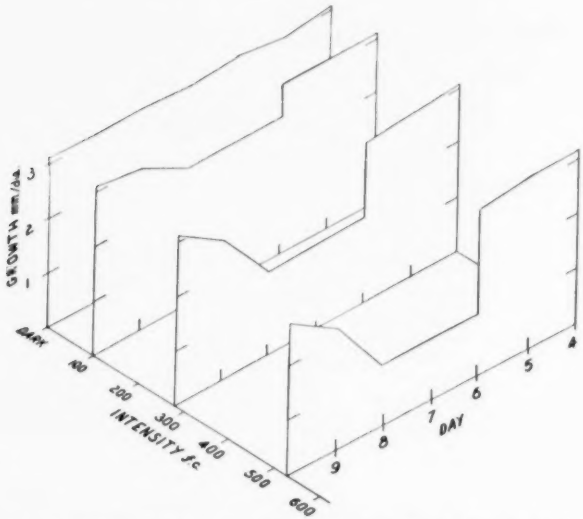


FIG. 2. The influence of light on growth (in mm diam. per day) of *P. herquei* Strain III. Days 1 to 4 (not shown), incubation period; days 4–6, dark period; days 6–8, light period at intensities indicated; days 8–10, dark period. Each point represents the average growth of ten colonies.

lamps served as a light source. The intensity was varied by changing the distance between the plates and the light source or by screening the light through gauze.

A number of experiments using the above procedure was conducted and gave similar results. The results of one typical experiment are shown graphically in FIG. 2.

REVERSIBILITY OF THE COLOR CHANGE: It was suspected in all of the previously conducted experiments dealing with the change in color from yellow to green that the living organism possessed the ability to

bring about a reversal of this reaction. Living and non-living colonies were exposed to light. After the colonies became green the plates were returned to the dark for 12 hours. The non-living colonies remained green while the living colonies became yellow. In another experiment actively growing colonies were exposed to light until they developed a green color. The plates were then wrapped in black paper and kept at 5°, 45°, and 25° C for a period of 12 hours. The colonies which were kept in the dark at 25° C became yellow, while the colonies which were kept in the dark at 45° and 5° C remained green.

INFLUENCE OF AMMONIA VAPOR: Exposure of yellow colonies of this organism to ammonia resulted in an almost instantaneous development of green color. However, exposure to the vapors of acetic acid would not bring about a reverse reaction. It is considered that the color reaction that occurs in this *Penicillium* is different from the one occurring in *Aspergillus flavus-oryzae* (**28**).

EXPERIMENTS IN VITRO

METHODS OF EXTRACTION: The material for experiments *in vitro* was prepared by the following methods. Yellow colonies approximately 30 to 35 mm in diameter were ground in a homogenizer with 20 ml of distilled water per colony. The material was centrifuged to remove the spores and the cellular debris. The supernatant was poured off into a clean test tube and the sediment discarded. Ammonium sulfate was added to the supernatant until a yellow precipitate formed. The material was again centrifuged. The supernatant was discarded and the precipitate resuspended in distilled water. The material obtained after this treatment was yellow in color and was capable of changing to green upon exposure to light.

A variation of the above method was to precipitate the material by adjusting the pH to 3 or 4 with 0.1 N. HCl. The material was resuspended by adjusting the pH to above pH 6 with the addition of 0.1 N. NaOH. It could also be washed several times using a buffer solution of approximately pH 4. The yellow solution obtained by this treatment was also capable of becoming green upon exposure to light.

Material obtained by the above methods was used in studying the requirements of the reaction. Tests were conducted in colorimeter tubes, and a mercury vapor lamp, or sunlight was used as a light source.

LIGHT AND OXYGEN REQUIREMENT: Tubes containing material obtained by ammonium-sulfate precipitation were purged with nitrogen, carbon dioxide, and oxygen, and exposed to light from a mercury vapor

lamp. Control tubes purged with the same gases were kept in the dark. The results are shown in TABLE II. The results indicate that light is accelerating an oxidation reaction.

INFLUENCE OF pH: For studying the influence of pH, buffer solutions were made up according to the formulations of Sørenson and Clark (**9**) and Lub. Standard acid and base solutions (**13**) were used for regulating the pH of the extract.

TABLE II

INFLUENCE OF OXYGEN AND LIGHT

	Light	Dark
Oxygen	+*	+**
Nitrogen	−	−
Carbon dioxide	−	−

(+) development of green color; (−) remain yellow; +* reaction occurred in less than 3 minutes; +** reaction occurred upon continued storage in the dark, 12 to 24 hours.

It was first determined that pH had no immediate influence upon the color change. For example the yellow extract remained yellow with the dropwise addition of 0.01 N. acid or 0.01 N. base. Furthermore, material extracted in buffer solutions of pH 3.3, 4.5, 5.1, 6.5, 7.5, and 8.6 for use in the following experiment also remained yellow.

The above mentioned buffer solutions were used to determine the optimum pH value for the reaction stimulated by light. The results are shown in TABLE III. It is interesting to note here that the pH range

TABLE III

INFLUENCE OF pH AND LIGHT

Buffer solution	3.3	4.5	5.1	6.5	7.5	8.6	9.9
Results	−	−	++	++	++	++	+

(−) no reaction; (++) strong reaction; (+) slight reaction.

which proved optimum for this reaction is almost identical to the pH range that proved optimum for maximum fluorescence of one of the pigments present in the organism. The above experiments were all repeated several times, occasionally using full strength buffer solutions; similar results were always obtained.

EXTRACTION AND CHARACTERIZATION

The green pigment was extracted with chloroform from either ammonium-sulfate-precipitated material or from whole green colonies. The material was first defatted with light petroleum and dried. The green

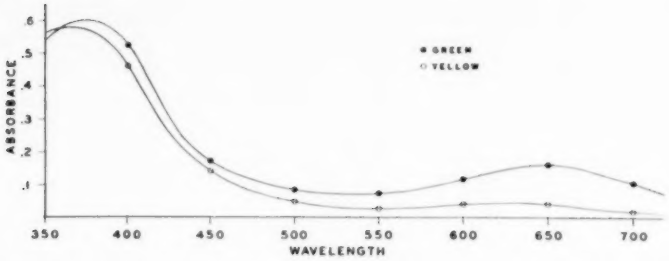


FIG. 3. Absorption spectra (mμ) of the green amorphous material in ethyl acetate: green, basic; yellow, acid.

chloroform solution was then washed repeatedly with water, both acid- and basic-buffered, until the wash water returned clear. The chloroform extract was then dried with magnesium sulfate, filtered, and evaporated to dryness. A green amorphous mass was obtained which could not be crystallized.

The green amorphous material when dissolved in ethyl acetate produced a green solution; however, upon addition of acid the color changed to yellow. The color change could not be reversed with addition of a base. The absorption spectra are shown in FIG. 3.

In the process of using various solvents to obtain the material in crystalline form it was discovered that nearly colorless, hygroscopic crystals formed upon evaporation of a pyridine solution of the green amorphous material. The crystals when dissolved in 95% ethyl alcohol gave a green solution. The solution remained green with the addition of acid; however, with the addition of base it became yellow. This color reaction was found to be reversable. The absorption spectra are shown

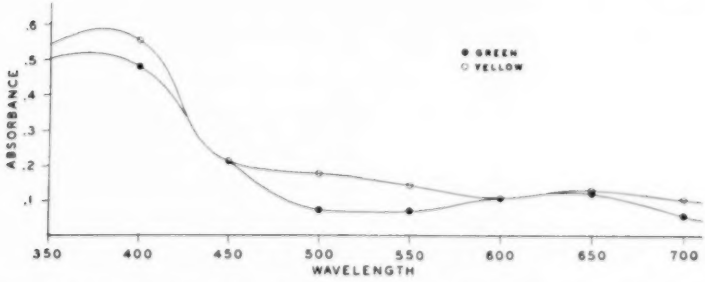


FIG. 4. Absorption spectra (mμ) of crystalline material from pyridine dissolved in 95% ethyl alcohol: green, acid; yellow, basic.

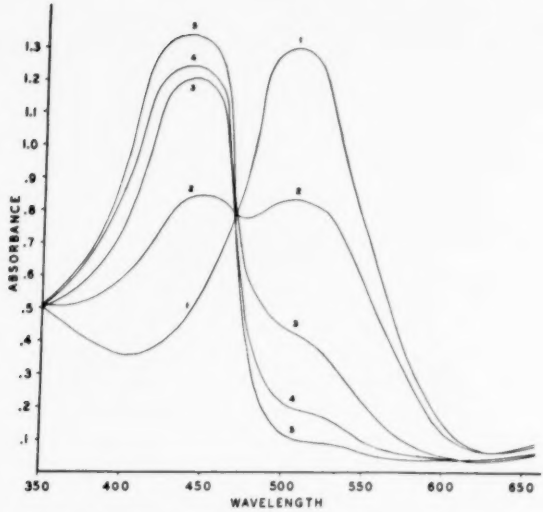


FIG. 5. Isohydric curves of the yellow-red indicator in ethyl alcohol (mμ). Curve 1: absorption in base, curves 2, 3, 4, and 5: absorption after addition of 1, 2, 3, and 4 drops of 0.01 N. HCl, respectively.

in FIG. 4. It is interesting to note that the colors produced by this material in acid and basic alcoholic solutions are reversals of those produced by the green amorphous material in the same solvent.

In attempting to heat the greenish amorphous material it was discovered that a fraction sublimed. Yellow, needle-like crystals sublimed at approximately 123° C at atmospheric pressure. This material when dissolved in 95% ethyl alcohol acted as a pH indicator. In acid solution it was yellow; in basic solution it was red. This color change was reversable. The isohydric curves (**6**) are plotted in FIG. 5.

TABLE IV

SUMMARY OF THE pH AND COLOR RELATIONSHIPS OF THE MATERIALS CONCERNED IN THIS SECTION

	pH 7.4	7.6	7.8	8.0	8.2	8.4	8.5	8.7
Yellow-red material	Yellow			Orange			Red	
Crystals from pyridine	Green			Yellow				
Green amorphous material	Yellow			Green				

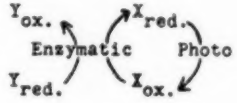
The color changes and the pH relationship of the compounds involved in this section are summarized in TABLE IV.

DISCUSSION

Experiments indicate at least three interactions of light on this organism, namely growth rate, color change and sporulation. At present, evidence indicates a correlation between two of these effects: the growth rate and the color change. The influence of light on growth parallels its influence upon the color change; *i.e.,* the higher the light intensity the greater the inhibition, and the deeper the intensity of the green color. Furthermore, when green colored colonies are returned to the dark they resume their maximum growth rate and at the same time revert to their original yellow color. These results, concerning the influence of light on growth rate, are in general agreement with those obtained by Porter and Bockstahler (**16**), Carlile (**8**), Tatarenko (**26**) and Zachariah *et al.* (**29**) with other fungi.

The influence of light on sporulation appears to be independent of its action on growth—as measured by radial extension. This assumption comes from the fact that the stimulatory effect on sporulation occurs at a different light intensity, and that above and below this intensity there is also an effect which results in the lack of sporulation. Organisms grown at 100 f. c. sporulated abundantly while above and below this intensity no sporulation occurred. These results are in agreement with those of Porter and Bockstahler (**16**), Johnson and Halpin (**14, 15**), Barnett and Lilly (**1**) and Tatarenko (**26**) working with other fungi.

Experiments *in vivo* and *in vitro* indicate that the color change; *i.e.,* yellow to green, is a photocatalytic oxidation. They also indicate that the active organism has the ability to reverse this reaction. The equation



might thus far be proposed for the reaction *in toto* (X, being the yellow-green compound). This implies that the yellow-green compound is the light acceptor and consequently that the reaction is of a primary process (**10, 23, 27**). There is, however, a number of additional factors which indicate intermediate reactions. For example, there is

present in the organism a fluorescent pigment (**7**). The pH range in which this pigment is most stable and in which it fluoresces most strongly is the same pH range at which the reaction occurs most rapidly. The implication here, then, is a photosensitized reaction reminiscent of photodynamic action (**4, 23**).

In all appearances the pigment involved in the color change differs from those already described for this organism by Sartory and Bainier (**21**), Burton (**7**), Harman *et al.* (**12**), Stodola *et al.* (**25**), Barton *et al.* (**2**), and Galarraga *et al.* (**11**). Further elucidation correlating the roles of the pigments in the organism with the action of light will, of course, depend upon quantitative studies.

SUMMARY

An investigation was made concerning the influence of light on a variant of *P. herquei.* It was found that light inhibited the vegetative growth of the organism. The influence appeared to be inversely proportional, *i.e.,* as light intensity increased, the radial extension of the mycelium decreased. It was also found that light exerted a profound influence upon the sporulation and the color of the organism. When grown in total darkness or at high light intensities, over 200 f. c., the organism failed to sporulate. However, it sporulated rather abundantly at 100 f. c.

Various aspects concerning the influence of light on the color of the organism were also investigated. It was found that yellow colonies when exposed to sunlight rapidly became green. The results indicated that the reaction was a photocatalytic oxidation, perhaps mediated by a fluorescent pigment. They also indicated that the living organism possessed the ability to reverse the color change, *i.e.,* green to yellow.

Various tests were conducted to determine the nature of the pigment complex. It appeared to be composed of at least two fractions. One fraction was not identified; the other fraction, obtained through sublimation, responded positively to tests for quinones and related compounds. It was found to be a pH indicator.

DEPARTMENT OF BIOLOGICAL SCIENCES
PURDUE UNIVERSITY
LAFAYETTE, INDIANA

BIBLIOGRAPHY

1. **Barnett, H. L. and V. G. Lilly.** 1950. Influence of nutritional and environmental factors upon asexual reproduction of *Choanephora cucurbitarium* in culture. Phytopath. **40**: 80–89.

2. **Barton, D. H. R., P. de Mayo, G. A. Morrison, W. H. Schaeppi and H. Raistrick.** 1956. Some observations on the constitutions of herqueinone and related compounds. Chem. and Ind. **1956**: 552–553.
3. **Birkinshaw, J. H.** 1953. Chemistry of the fungi. Ann. Rev. Biochem. **22**: 371–398.
4. **Blum, H. F.** 1941. Photodynamic Action and Diseases Caused by Light. Reinhold, New York.
5. **Bracken, A.** 1955. The Chemistry of Micro-organisms. Pitman, New York.
6. **Brode, W. R.** 1924. The determination of hydrogen-ion concentration by a spectrophotometric method and the absorption spectra of certain indicators. Jour. Am. Chem. Soc. **46**: 581–596.
7. **Burton, H. S.** 1949. Antibiotics from penicillia. Brit. Jour. Exp. Pathol. **30**: 151–158.
8. **Carlile, M. J.** 1956. A study of the factors influencing non-genetic variation in a strain of *Fusarium oxysporum*. Jour. Gen. Microbiol. **14**: 643–654.
9. **Clark, W. M.** 1928. The Determination of Hydrogen Ions. Williams and Wilkins, Baltimore.
10. **Ellis, C., A. A. Wells and F. F. Hegroth.** 1941. The Chemical Action of Ultraviolet Rays. Reinhold, New York.
11. **Galarraga, J. A., K. G. Neill and H. Raistrick.** 1955. The coloring matters of *Penicillium herquei* Bainier and Sartory. Biochem. Jour. **61**: 456–464.
12. **Harman, R. E., J. Cason, F. H. Stodola and A. L. Adkins.** 1955. Structural features of herqueinone, a red pigment from *Penicillium herquei*. Jour. Org. Chem. **20**: 1260–1269.
13. **Lange, N. A. and Forker, G. M.** 1956. Handbook of Chemistry. Ninth Edition. Handbook Publishers, Sandusky.
14. **Johnson, T. W. and J. E. Halpin.** 1954. Environmental effects on conidial variation in some Fungi Imperfecti. Jour. Elisha Mitchell Sci. Soc. **70**: 314–326.
15. —— **and** ——. 1952. Influence of light on the morphology and production of conidia in some species of Dematiaceae. Phytopath. **42**: 342.
16. **Porter, C. L. and H. W. Bockstahler.** 1928. Concerning the reaction of certain fungi to various wave lengths of light. Proc. Ind. Acad. Sci. **38**: 133–135.
17. **Raistrick, H.** 1940. Biochemistry of the lower fungi. Ann. Rev. Biochem. **9**: 571–592.
18. **Raper, K. B. and C. Thom.** 1949. A Manual of the Penicillia. Williams and Wilkins, Baltimore.
19. **Riedhart, J. M.** 1957. *Cover photograph*. Amer. Biol. Teacher, Jan.
20. **Riedhart, J. M. and C. L. Porter.** A unique color reaction of a *Penicillium* sector. (Abs.) Proc. Ind. Acad. Sci. **66**: 68. 1957.
21. **Sartory, A. and G. Bainier.** 1911. Sur un *Penicillium* nouveau à propriétiés chromogènes singulières. Soc. Biol. Compt. Rend. **71**: 229–230.
22. **Smith, George.** 1954. The pigments of the lower fungi (molds). Rapp. et Commun. VIII Congr. Int. Bot. Paris **18–20**: 83–89.
23. **Stacy, R. W., D. Williams, R. Worden and R. McMorris.** 1955. Essentials of Biological and Medical Physics. McGraw-Hill, New York.

24. **Stauffer, J. F. and M. P. Backus.** 1954. Spontaneous and induced variation in selected stocks of the *Penicillium chrysogenum* series. Ann. N. Y. Acad. Sci. **60**: 35–49.

25. **Stodola, F. H., K. B. Raper and D. I. Fennell.** 1956. Pigments of *Penicillium herquei*. Nature **167**: 773–774.

26. **Tatarenko, E.** 1954. [The influence of light on the development of mould fungi.] Microbiology, Moscow. Summarized in Rev. Appl. Mycol. **34**: 803. 1955.

27. **Taylor, H. A.** 1957. Photochemical reactions leading to unstable species. Ann. N. Y. Acad. Sci. **67**: 477–484.

28. **Thom, Charles.** 1954. The evolution of species concepts in *Aspergillus* and *Penicillium*. Ann. N. Y. Acad. Sci. **60**: 24–34.

29. **Zachariah, A. T., H. N. Hansen and W. C. Snyder.** 1956. The influence of environmental factors on cultural characters of *Fusarium* species. Mycologia **48**: 459–467.

NEW WATER MOLDS FROM FLORIDA[1]

A. W. ZIEGLER

(WITH 16 FIGURES)

Two new species of fungi belonging to the family Saprolegniaceae have been isolated from water collections made during a survey of the water molds of the state of Florida.

Achlya mucronata sp. nov.

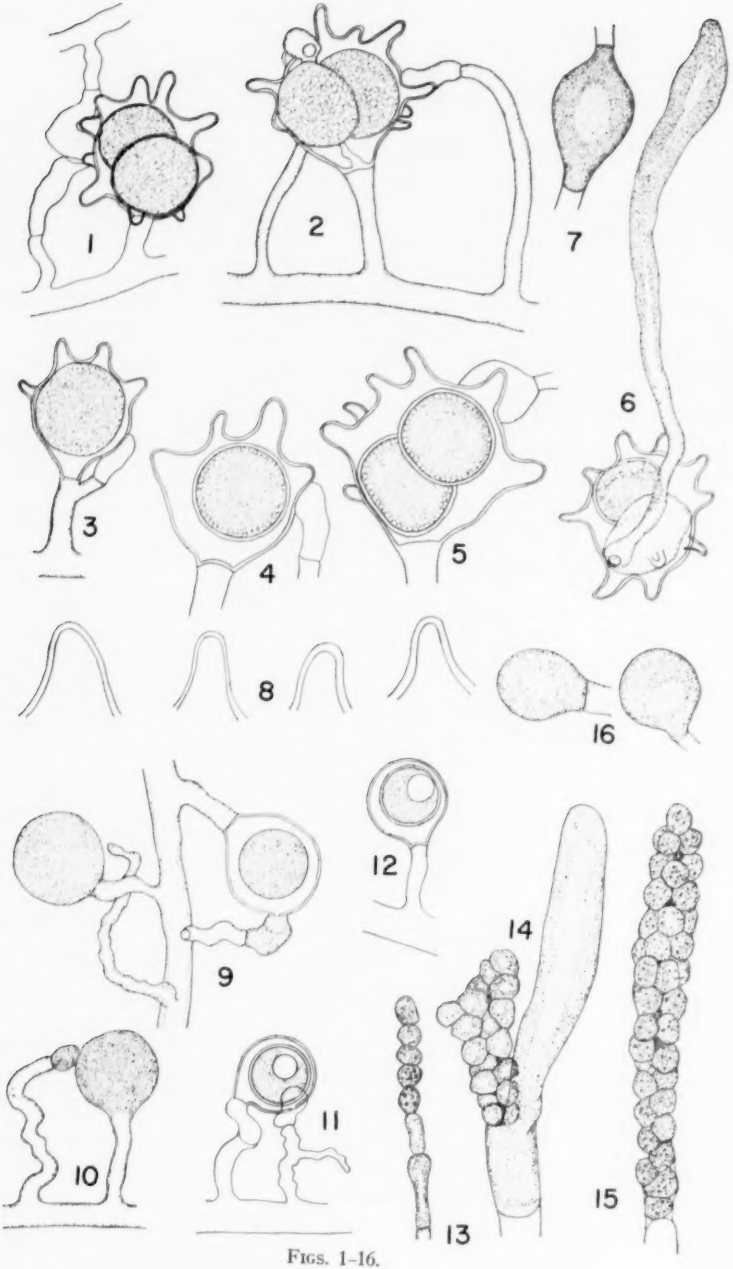
Sporangia copiosa in culturis juvenibus. Ejectio sporarum typica generis. Oogonia copiosa, globosa, 29–66 μ in diam., plerumque 33–51 μ, excl. papillis. Papillae obtusae, parum recurvae, plerumque 14 μ longae. Ova subcentrica, singula, raro plura, 22–44 μ in diam., plerumque 26–37 μ, non complentia oogonium. Antheridia copiosa, plerumque origine monoclina (60%) sed interdum androgyna et diclina, omnia vacua antequam ova matura sunt.

Mycelium moderately dense. Zoosporangia formed only in very young cultures, long cylindrical, 106–127 × 15–19 μ. Gemmae terminal, resembling small non-papillate oogonia. Oogonia abundant, spherical; lateral on stalks of various lengths; with blunt tapering and usually slightly laterally recurved papillae, about 14 μ in length; 29–66 μ, predominantly 33–51 μ in diameter, exclusive of the papillae. Oospores subcentric, single, rarely more, 22–44 μ, predominantly 26–37 μ in diameter, not filling the oogonium. Antheridial branches usually monoclinous (60%) but also androgynous and diclinous, developing at oospore-formation stage and persisting only until zygotes are mature. Mature zygote germinating by an unbranched germ tube bearing a terminal zoosporangium.

From water, collected in ditch in Gulf County, Florida, collection No. *222*, November 17, 1955, TYPE. Also collected in water from a small stream, Walton County, Florida, collection No. *280*, December 1, 1955.

This new species is similar to *Achlya radiosa* (**1**, **5**) which has not been found in this country. It differs from that species, however, in its smaller oogonia, type of papillate ornamentations (FIGS. 1–6, 8), an-

[1] This work was made possible by grant No. 2013 from the National Science Foundation. The author wishes to thank Dr. Leland Shanor and Dr. T. W. Johnson, Jr., for their help and advice in preparing the manuscript.



FIGS. 1–16.

theridial origin, production of zoosporangia, and its failure to discharge spores in an amorphous mass. FIG. 8 shows large-scale drawings of the papillae of *A. mucronata*. They are blunt at the tip with one slightly recurving side as opposed to the mammiform ornamentations found in *A. radiosa*. The characteristics of *A. mucronata* make it easily separable from other species with subcentric oospores (1, 3, 4).

Limited studies using different food sources, variable temperature, and buffer solutions have demonstrated that the taxonomic criteria remain unchanged regardless of environment.

Brevilegnia globosa sp. nov.

Zoosporangia copiosa in omnibus culturis, magnitudine varia. Emissio sporarum varia sicut in generibus *Brevilegnia, Thraustotheca,* et *Dictyucho*. Oogonia copiosa, globosa, 22–32 μ in diam., plerumque 29 μ. Longitudo caulis oogoniorum plerumque aequalis diametro oogoniorum. Ova eccentrica, spherica, semper singula, plerumque 28 μ in diam., non complentia oogonium. Antheridia monoclina et plerumque parum torta.

Mycelium moderately dense, reaching a diameter of 14 mm on hemp seed. Zoosporangia very abundant in both old and young cultures, long, cylindrical; variable, emerging as in the genera *Brevilegnia, Thraustotheca,* and *Dictyuchus*. Gemmae ovoid, terminal, often intercalary. Oogonia plentiful, spherical; lateral on stalks $\frac{1}{2}$–2 times the diameter of oogonium; 22–31 μ, mostly 29 μ, in diameter. Oospores eccentric, spherical, single, 22–33 μ, predominantly 28 μ; not filling the oogonium. Antheridial branches monoclinous; usually slightly contorted; antheridial cells apically appressed. Mature oospore germinating after 3 months by a germ tube with a terminal zoosporangium; spore behavior as in *Brevilegnia*.

From water collected in small stream, Gadsden County, Florida, collection No. *15*, September 27, 1955, TYPE.

Brevilegnia globosa is notable for its varied spore behavior. At times the spores are discharged in the manner of *Thraustotheca* and, at other times, as in *Brevilegnia* and *Dictyuchus*. On two occasions the achlyoid type of spore discharge has been seen. A study of the descrip-

FIGS. 1–8. *Achlya mucronata*. 1. Oogonium with monoclinous and diclinous antheridia. 2. Oogonium with monoclinous antheridium. 3. Oogonium with androgynous antheridium. 4, 5. Oogonia with mature subcentric oospores. 6. Oospore germinating. 7. Gemma. 8. Outline drawings of typical papillae. FIGS. 9–16. *Brevilegnia globosa*. 9. Oogonium with developing oospore and contorted monoclinous antheridium. 10. Oogonium with developing antheridium. 11, 12. Mature oogonia. 13, 14, 15. Zoosporangia. 16. Gemmae. All figures $\times$760 except 8, which is $\times$1900. Figures reduced about 2$\frac{1}{4}$ times.

tive literature of this genus reveals that spore behavior may be quite varied. To substantiate this new species, asexual characteristics because of their variability must be disregarded for diagnostic purposes.

This new species differs from *B. crassa* (**6**), *B. subclavata*, *B. linearis*, and *B. bispora* (**1**, **2**, **3**) in its larger oogonia and oospores, and from the latter by the monoclinous antheridial origins. It may be separated from *B. megasperma* var. *brevicaulis* (**6**) in its smaller oospores, and more slender oogonial stalks. *B. globosa* is distinct from other species (**4**, **6**) with its monoclinous antheridia, contorted antheridial branches, and regular wall thickness.

In 1942 Salvin (**7**) proposed reducing the genus *Brevilegnia* to a subgenus in the genus *Thraustotheca*. At that time, his proposal perhaps was premature. It will be noted, however, that for most of the species described since that time, the variability of zoospore behavior is mentioned. This author does not agree with Salvin's proposal to place all species of *Brevilegnia* except *T. bispora* under *T. unisperma*. Too many of the new species are stable organisms and are deserving of species status. However, the characters which distinguish the genus are no longer valid in view of recent work. It is this author's opinion that the genus *Brevilegnia* should be reduced to a subgenus of *Thraustotheca* and new keys for the identification of the species constructed.

DEPARTMENT OF BIOLOGICAL SCIENCES, DIVISION OF BOTANY
FLORIDA STATE UNIVERSITY
TALLAHASSEE, FLORIDA

LITERATURE CITED

1. **Coker, W. C.** 1923. The Saprolegniaceae. Univ. of N. C. Press. Chapel Hill.
2. ——. 1927. Other water molds from the soil. Jour. Elisha Mitch. Sci. Soc. **42**: 207–226.
3. —— **and V. D. Matthews.** 1937. Saprolegniales. North Amer. Flora **2** (1): 15–67.
4. **Johnson, T. W., Jr.** 1950. A study of an isolate of *Brevilegnia* from New Caledonia. Mycologia **42**: 242–252.
5. ——. 1956. The Genus *Achlya*. Univ. of Michigan Press. Ann Arbor.
6. **Rossy-Valderrama, C.** 1956. Some water molds from Puerto Rico. Jour. Elisha Mitch. Sci. Soc. **72**: 129–137.
7. **Salvin, S. B.** 1942. Variations of specific and varietal character induced in an isolate of *Brevilegnia*. Mycologia **34**: 38–51.

STUDIES OF SOME TREMELLACEAE. II. THE GENUS DUCTIFERA [1]

KENNETH WELLS [2]

(WITH 2 FIGURES)

Tremella pululahuana Pat. was described in 1893 from a specimen collected in Ecuador. This epithet was later transferred by Bourdot and Galzin (Hymén. Fr. 48. 1928) to the genus *Bourdotia*. These authors considered *T. pululahuana* as being composed of three subspecies, *Bourdotia pululahuana* ssp. *pululahuana*,[3] *B. pululahuana* ssp. *galzinii* (Bres.) Bourd. & Galz. and *B. pululahuana* ssp. *caesia* (Bres. & Torr.) Bourd. & Galz. Rogers (Univ. Iowa Stud. Nat. Hist. **17** (1): 38–39. 1935) regarded Patouillard's species as conspecific with *Sebacina galzinii* Bres. and *Bourdotia caesia* Bres. & Torr. and reduced the two latter names to later synonyms under the combination *Sebacina pululahuana* (Pat.) Rogers. McGuire (Lloydia **4**: 33–35. 1941), following his examination of the type, concluded that *T. pululahuana* was not conspecific nor congeneric with *S. galzinii* and included in the latter species *Bourdotia pululahuana* in the sense of Bourdot and Galzin and *Sebacina pululahuana* in the sense of Rogers.

Lloyd (Myc. Writ. **5**. Myc. Notes 45: 629–630. 1917), following an examination of the type of *Seismosarca hydrophora* Cooke, concluded that this species was congeneric with *Exidiopsis alba* Lloyd and accordingly transferred *E. alba* to the genus *Seismosarca*. The same year, Lloyd (Myc. Writ. **5**. Myc. Notes 50: 710. 1917) described the genus *Ductifera* to include a single species, *D. milleii*. He was unable to demonstrate septate basidia of any type, but clearly indicates that this

[1] Portion of a thesis submitted to the Graduate College of the State University of Iowa in partial fulfillment of the requirements for the degree of Doctor of Philosophy, under the direction of Professor G. W. Martin. The first paper in this series was published in Lloydia **20**: 43–65. 1957.

[2] Present address: Department of Botany, University of California, Davis, California.

[3] Bourdot and Galzin apparently regarded Patouillard's species as being represented in France by two subspecies, which were distinct from the Ecuadorian form; therefore, it is necessary to imply a third subspecies in order to conform to the modern system of nomenclature.

species is to be included in the Auriculariaceae, apparently regarding the immature gloeocystidia as auriculariaceous probasidia.[4] Examination of the type specimen of *D. milleii* (C. G. Lloyd 33578) reveals the presence of numerous cruciately-septate basidia, and gloeocystidia which become filled with a yellow-granular substance at maturity. The microscopic and macroscopic characters indicate that the specimen is to be included within the concept of *T. pululahuana*. Martin (Mycologia **43**: 112–113. 1951) has concluded that *Seismosarca hydrophora* Cooke, the type species of the genus *Seismosarca*, is not a member of the Tremellaceae and suggests that the genus *Seismosarca* be discarded; therefore, it is necessary to adopt *Ductifera* as the generic name, even though Lloyd was obviously confused as to the true affinities of *D. milleii*.

Olive (Bull. Torrey Bot. Club **81**: 334. 1954) reported that the species described by Viégas (Bragantia **5**: 243. 1945) as *Seismosarca stratosa* Viégas was more closely related to species included by McGuire (Lloydia **4**: 32–42. 1941) in the section *Bourdotia* of the genus *Sebacina* than to species included in the genus *Seismosarca* (sensu Lloyd). I (Lloydia **20**: 56. 1957) should regard the type of Viégas's species as a *Bourdotia;* however, in my opinion, it is best included within the concept of *Bourdotia cinerea* (Bres.) Bourd. & Galz. Olive has described two species with characters similar to *D. milleii*. The description of *Seismosarca tomentosa* Olive is apparently the first recognizable account of a distinct species; however, the name is predated by *Tremella micropera* Kalchbr. & Cooke. The other, *Exidia cystidiata* Olive, is, in my opinion, best referred to a species described by Möller (Protobas. 169. 1895) as *Exidia sucina* Möll. A third species originally described by Olive (Bull. Torrey Bot. Club **78**: 110. 1951) as *Seismosarca cartilaginea* Olive is considered here as an excluded species because of the texture of the fructifications and the difficulty of being able to demonstrate tremellaceous basidia clearly in the type collection. Specimens of *Ductifera* which have been referred by Lloyd and other authors to *S. hydrophora* are, in most cases, best included in *E. sucina*.

Sebacina (*Bourdotia*) *megaspora* Martin is included in the genus *Ductifera* since the margins of the fructifications are abrupt and free, there is an ascending layer of distinct hyphae, and the basidia are not formed in a close acropetal series as is characteristic of the genus *Bourdotia*.

Ervin (Mycologia **48**: 692. 1956) established the genus *Gloeotromera* based on *Exidiopsis alba* Lloyd to include those tremellaceous

[4] Basidial terminology used in this paper is as defined by Martin (Brittonia **9**: 25–30. 1957).

fungi which have a cerebriform to convolute fructification and have gloeocystidia present in the hymenium. She treated two species, *G. alba* and *G. pululahuana,* which are differentiated on the basis of texture,

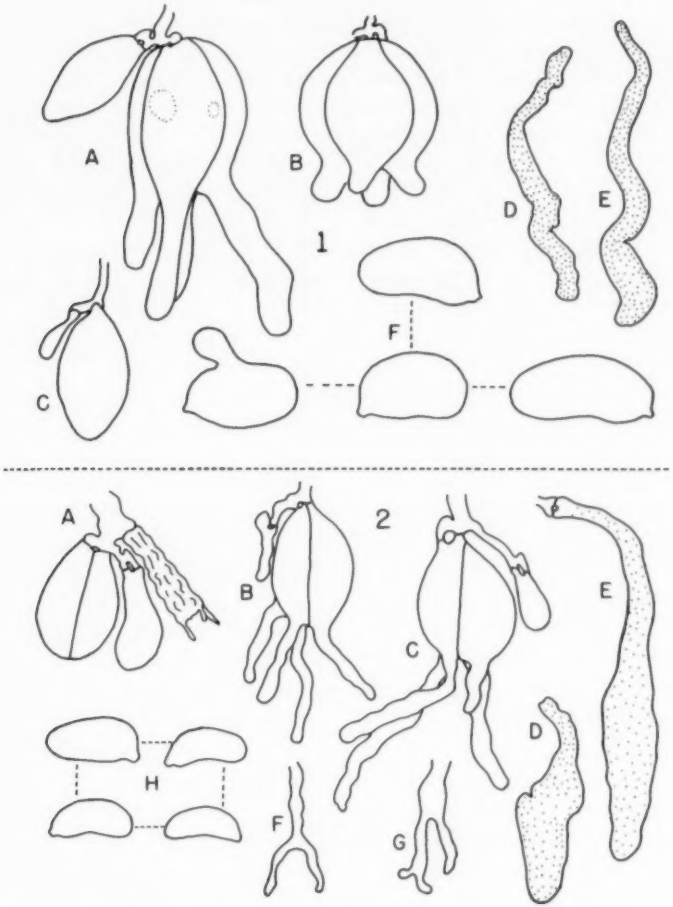


FIG. 1. *Ductifera megaspora.* A–C. Basidia in various stages of development, × 875 (*J. B. Cleland 43*). D, E. Gloeocystidia, × 875 (D from *JBC 39;* E from *JBC 24*). F. Basidiospores, one germinating, × 875 (*JBC 24*). FIG. 2. *Ductifera micropera.* A–C. Basidia, × 1000. D, E. Gloeocystidia × 1000. F, G. Tips of dikaryophyses, × 1000. H. Basidiospores, × 875 (*MacOwan 1351*). All figures drawn with the aid of a camera lucida.

color, and basidiospore characters. I (Lloydia **20**: 58. 1957) have been unable to verify this distinction and would include *G. alba* within the concept of *Tremella pululahuana*.

The genus *Ductifera* may be distinguished from *Exidia glandulosa* Fries and related species by the presence of gloeocystidia and the lack of the tough epihymenial layer in the former group. These two groups are similar in manner of growth, internal structure, and basidial structure; however, since a clear distinction apparently exists between the two groups and since the species here included in *Ductifera* are quite closely related, the genus *Ductifera* seems worthy of recognition.

The differentiating characters between the genera *Bourdotia* and *Ductifera* are more sharply defined. The distinct hyphae with elaborate clamp-connections and which form definite ascending hyphal layers in the genus *Ductifera* are not found in any species of *Bourdotia*. In addition there is a distinction between the two genera in the manner of growth; i.e., in *Bourdotia* the margins are adnate and indeterminate, whereas in *Ductifera* the margins are free and determinate.

Any hypothesis regarding the phylogenetical relationships of these species, with the exception of a possible origin from a *Bourdotia*-like ancestor, must be viewed as extremely speculative. If this genus is considered as having evolved from *Bourdotia*, then *E. sucina* should be regarded as the most primitive species, since in this species the characters of the gloeocystidia and dikaryophyses are more similar to certain species of *Bourdotia* than are those of other species here included in *Ductifera*.

The method of study used was as defined by Martin (Univ. Iowa Stud. Nat. Hist. **19** (3): 9. 1952). It was found convenient to supplement the Phloxine with an equal amount of 1% aqueous solution of Congo Red.

Unless otherwise indicated, portions of the specimens examined are deposited in the Mycological Herbarium of the State University of Iowa. If located elsewhere the respective herbaria are indicated by the symbols recommended by Lanjouw and Stafleu (Index herbariorum. Part I. The herbaria of the world. Ed. 3. Utrecht. 1956).

I should like to express my appreciation to Dr. G. W. Martin for his many helpful suggestions and criticisms given during the course of this and other studies. Appreciations are also due Dr. C. J. Alexopoulos, Dr. L. S. Olive, Dr. D. P. Rogers, and Dr. F. T. Wolf for their aid in securing several specimens.

DESCRIPTION OF GENUS

DUCTIFERA Lloyd, Myc. Writ. **5**. Myc. Notes 50: 711. 1917.
Seismosarca Cooke sensu Lloyd, Myc. Writ. **5**. Myc. Notes 45: 629. 1917
Gloeotromera Ervin, Mycologia **48**: 692. 1956.

Soft to firm waxy-gelatinous, arising as small pustules which become confluent to form pulvinate, cerebriform, or convolute fructifications; subhymenial hyphae distinct, with elaborate clamp-connections throughout, and forming a definite ascending layer; hymenium inferior or unilateral; dikaryophyses present, branching, and forming a well-defined layer above the basidia; gloeocystidia at first hyaline, becoming yellow-granular and flexuous at maturity, subcylindrical to subclavate, restricted to the hymenium; basidia subglobose to clavate, subtended by clamp-connections, becoming 2–4-celled by longitudinal or oblique septa; basidiospores cylindrical-curved, hyaline, germinating by repetition.

TYPE: *Ductifera milleii* Lloyd.

KEY TO SPECIES

a. Gloeocystidia generally restricted to region above basidia; basidiospores 20–30 × 10–15 μ.....1. *D. megaspora*
a. Gloeocystidia arising at the same level as or below the basidia; basidiospores usually smaller than 20 × 10 μ.....b
b. Dikaryophyses usually abundant and much-branched; gloeocystidia often exceeding 80 μ in length; basidiospores 9–12.5 × 4.5–6 μ.....2. *D. pululahuana*
b. Dikaryophyses relatively sparse and simple to little-branched; gloeocystidia rarely exceeding 80 μ in length.....c
c. Fructification at first pustular, soon becoming confluent and cerebriform to somewhat convolute; soft to waxy-gelatinous; basidiospores 10–15 × 6–9 μ.....3. *D. sucina*
c. Fructification pustular and erumpent; firm waxy-gelatinous; margins often white-tomentose; basidiospores 16–21 × 5.5–7 μ.....4. *D. micropera*

ACCEPTED SPECIES

1. **Ductifera megaspora** (Martin) comb. nov.
Sebacina megaspora Martin, Mycologia **28**: 214. 1936.

Soft waxy-gelatinous, effused in small patches which become confluent to form fructifications up to several millimeters in extent, somewhat cerebriform, dark grayish-brown to dingy drab, semitranslucent; surface undulate to tuberculate; margins abrupt and free; drying to a blackish-brown crust; fructification 400–1500 μ thick, consisting of an ascending layer arising from the substrate, interwoven and branching, distinct, with elaborate clamp-connections throughout, 1.5–4(–5.5) μ in

diameter, terminating in a hymenium of gloeocystidia, dikaryophyses, and fertile hyphae; gloeocystidia abundant, primarily restricted to zone above the near-mature basidia, subclavate or subcylindrical, at first hyaline, soon becoming yellow-granular and flexuous, 30–70(–100) × (3.5–) 5–7.5(–8.5) μ; fertile hyphae with clamp-connections throughout, 2.5–4 μ; probasidia at first broadly ovate, subtended by clamp-connections, becoming subglobose and longitudinally septate into 2 or 4 cells, guttulate, 25–35 × 18–30 μ; epibasidia tubular and somewhat flexuous, guttulate, relatively uniform in diameter, up to 120(–150) μ in length and 4.5–7 μ in diameter; basidiospores usually guttulate, cylindrical-curved or elliptical and laterally depressed, with conspicuous blunt apiculi, 20–30(–38) × 10–15(–16) μ, germinating by repetition.

On rotten wood. Known only from South Australia.

Type locality: South Australia, National Park.

Illustration: Mycologia **28**: 215, *f. 1.*

Specimens examined: South Australia: National Park, *J. B. Cleland 43* (TYPE); Mt. Lofty, *J. B. Cleland 24, 39.*

D. megaspora is well characterized by the large globose basidia, the large usually cylindrical-curved basidiospores, and the numerous clavate gloeocystidia which are generally restricted to the zone above the basidia. The gloeocystidia are the smallest within the genus.

This species is transferred to the genus *Ductifera* because of the basal hyphal layer of distinct interwoven hyphae arising directly from the substrate, the abrupt, somewhat free margins, and the cerebriform macroscopic aspect. As in the other members of the genus, the fertile hyphae and gloeocystidia of *D. megaspora* arise from a loosely interwoven hyphal layer and not, as in the genus *Bourdotia,* from a dense horizontal hyphal layer.

2. **Ductifera pululahuana** (Pat.) comb. nov.

Tremella pululahuana Pat., Bull. Soc. Myc. Fr. **9**: 138. 1893.

Ductifera milleii Lloyd. Myc. Writ. **5**. Myc. Notes 50: 711. 1917.

Bourdotia pululahuana (Pat.) Bourd. & Galz. ssp. *pululahuana,* Bourd. & Galz., Hymén. Fr. 48. 1928.[3]

Following additional study of several specimens, it appears unlikely that the basidia of this species become separated into apical fertile portions and basal stalks by the formation of secondary septa as previously reported (Lloydia **20**: 59. 1957). In addition, I neglected to mention the excellent photograph of a specimen of this species presented by Olive (Bull. Torrey Bot. Club **80**: 37, *f. 19*). For further discussion of this species see Lloydia **20**: 58–62. 1957.

Additional specimens examined: Indiana: Turkey Run State Park, *R. J. Bandoni*, 18 May, 1957 (3 collections). Tennessee: *F. T. Wolf 679, 716* (in VDB). Panamá: Prov. Chiriquí, *G. W. Martin 2478*. Ecuador: C. G. Lloyd 33578 (TYPE of *Ductifera milleii* Lloyd, in BPI). Argentina: *R. Singer T-717*.

3. **Ductifera sucina** (Möll.) comb. nov.
Exidia sucina Möll., Protobas. 169. 1895.
Sebacina lactescens Burt, Ann. Missouri Bot. Gard. **13**: 336. 1926.
Exidia cystidiata Olive, Bull. Torrey Bot. Club **81**: 333. 1954.
Gloeotromera sucina (Möll.) Wells, Lloydia **20**: 62. 1957.

The type collection of *Exidia cystidiata* Olive has a soft-gelatinous texture with a relatively loose arrangement of the subhymenial hyphae, clavate gloeocystidia which rarely exceed 80 μ in length, and relatively large basidiospores. These characters are found in the other specimens which have been assigned to this species. An illustration not previously noted is presented by Olive in Bull. Torrey Bot. Club **81**: 330, *f. 13–22*; 337, *f. 52a*.

As stated earlier, it appears that Möller's specific epithet provides the earliest known name available for this species; therefore, it is necessary to treat *Sebacina lactescens* Burt and *Exidia cystidiata* Olive as later synonyms.

Additional specimens examined: North Carolina: Highlands, *L. S. Olive*, 17 July, 1953 (TYPE of *Exidia cystidiata* Olive, in NY). Panamá: Prov. Chiriquí, *G. W. Martin 2521*.

4. **Ductifera micropera** (Kalchbr. & Cke.) comb. nov.
Tremella micropera Kalchbr. & Cke., Grevillea **9**: 18. 1880.
Seismosarca tomentosa Olive, Mycologia **39**: 99. 1947.
Exidia tomentosa (Olive) Olive, Bull. Torrey Bot. Club **81**: 333. 1954.

Firm waxy-gelatinous, appearing as small erumpent pustules up to 2–8 mm in diameter and 1–2 mm in thickness, sometimes becoming confluent, white to pale brown when moist; surface of larger fructifications strongly tuberculate; margins free and determinate, sometimes appearing as a white tomentose border which is composed of radiating strands of hyphae; drying to dark ochraceous, pulvinate masses; fructifications consisting of a basal layer of densely interwoven hyphae, from which arises an ascending layer of branching, loosely interwoven, distinct hyphae, 1.5–4(–7) μ, with elaborate clamp-connections throughout, terminating in a hymenium of fertile hyphae, dikaryophyses, and gloeocystidia; ascending layer sometimes arising directly from the substrate;

mineral accretions scattered throughout the fructification; gloeocystidia cylindrical to subclavate or subfusiform, at first hyaline, becoming yellow-granular and flexuous, 25–75 × 4.5–13.5 μ, dikaryophyses simple to little-branched, nodulose, 1–3.5(–4.5) μ, forming a layer 20–50 μ above the near-mature basidia; fertile hyphae forming turgid basidia in a zone 25–100 μ wide, 2–4(–7) μ in diameter; basidia subtended by well-defined clamp-connections, arising as elongate to clavate structures, becoming 2–4-celled by the formation of vertical to oblique septa, often guttulate, 14–21(–27) × 9–13.5(–15.5) μ; epibasidia tubular, enlarging somewhat towards the apex, up to 100(–130) μ in length, 1.5–3 μ in diameter; basidiospores cylindrical-curved, with blunt apiculi, 15–21 × 5.5–7.5 μ.

On corticated limbs. Known only from Georgia and South Africa.

Type locality: Somerset East, South Africa.

Illustrations: Grevillea **9**: *pl. 135, f. 1(a–d)*; Mycologia **39**: 97, *f. 6*.

Specimens examined: South Africa: *MacOwan 1351* (TYPE, in K). Georgia: Athens, *L. S. Olive*, 15 Nov., 1945 (TYPE of *Seismosarca tomentosa* Olive).

The internal structure of *D. micropera* is quite similar to *D. sucina;* however, the subhymenial hyphae and dikaryophyses in *D. micropera* are more distinct and closely arranged. In addition, there is a distinguishable difference in texture and basidiospore size between the two species. Macroscopically the type of *D. micropera* may be distinguished from specimens included in *D. sucina* by the pustular, erumpent aspect of the former species.

Although Olive's description of *Seismosarca tomentosa* represents the first accurate and complete description of this species, the type of *Tremella micropera* is sufficiently similar to the type of *S. tomentosa* to be included in the same species. MacOwan's collection of this species is somewhat more mature than Olive's collection; however, the internal structure of the two specimens is essentially similar. Macroscopically, the type *S. tomentosa* has several fructifications which have a white tomentose border, whereas this character is lacking in the type of *T. micropera*. This difference is, in my opinion, due to the more advanced stage of maturity of the latter collection. The hyphae composing the white tomentose border apparently constitute the laterally actively growing edge of the fructification. Since, as Olive points out for his collection, very young fructifications are entirely covered with these loosely arranged, radiating hyphae, they are, as a result, entirely white-tomentose in aspect. In older specimens, the white-tomentose character is restricted to the margins or entirely absent.

In the illustrations and description of this species by Kalchbrenner and Cooke basidiospores which develop three septa are indicated; how-

ever, I was unable to observe this character in the type collection or in any other specimen which has been properly referred to the genus *Ductifera*.

I am indebted to Dr. R. J. Bandoni for bringing to my attention the type specimen of *Tremella micropera* Kalchbr. & Cke.

SPECIES DUBIA

1. *Ductifera rosea* Rick, Egatea **18**: 345. 1933.

Authentic specimens of this species have not been examined; therefore, its affinities are unknown.

SPECIES EXCLUDENDAE

1. *Exidia fragilis* (Olive) Olive, Bull. Torrey Bot. Club **81**: 333. 1954.
Seismosarca cartilaginea Olive, Bull. Torrey Bot. Club **78**: 110. 1951. Not *Exidia cartilaginea* Lundell & Neuh.

The type collection of this species dries to a dark, horny, ochraceous mass which is quite difficult to revive. The texture after soaking is cartilaginous. Numerous microscopic examinations have revealed the presence of large globose cells, near the apparent upper surface, from which hyphal strands project in various directions. These structures may or may not become irregularly septate, do not stain internally with Phloxine, and are not formed in a definite zone; therefore, these structures are not, in my opinion, basidia. The texture, internal structure, and the characters of the hyphae suggest *Auricularia* or a related genus; however, since it was impossible to demonstrate basidia of any type, the true affinities of this collection are not known with certainty. Certainly this species is not to be included within the genus *Ductifera*.

Specimen examined: South Carolina: Jocassee, *L. S. Olive*, 5 July, 1950 (TYPE).

2. *Seismosarca stratosa* Viégas, Bragantia **5**: 243. 1945.
Sebacina stratosa (Viégas) Olive, Bull. Torrey Bot. Club **81**: 334. 1954.

The type of this species should, in my opinion, be referred to *Bourdotia cinerea* (Bres.) Bourd. & Galz. (Lloydia **20**: 58. 1957).

SUMMARY

1. The generic name *Ductifera* Lloyd is proposed as the valid designation for those species which have been included previously in *Seismo-*

sarca Cooke sensu Lloyd or *Gloeotromera* Ervin. Four species are recognized as belonging to the genus; i.e., *D. megaspora, D. pululahuana, D. sucina,* and *D. micropera.* Since these species are regarded as being closely related and appear to be quite distinct from *Exidia glandulosa* Fries and related species, the genus *Ductifera* is treated as distinct. Authentic specimens of *D. rosea* Rick have not been examined; therefore, the affinities of this species are unknown. *Exidia cartilaginea* (Olive) Olive and *Seismosarca stratosa* Viégas are treated as excluded species.

2. The genus is characterized by tremellaceous basidia; cylindrical-curved basidiospores; elaborate clamp-connections in the subhymenial hyphae; determinate, pustular fructifications; and soft to firm waxy-gelatinous texture.

3. The species of *Ductifera* are known from South Africa, Australia, and North and South America.

DEPARTMENT OF BOTANY
STATE UNIVERSITY OF IOWA
IOWA CITY, IOWA

A PRELIMINARY SURVEY OF THE GYMNOASCACEAE. I.

HAROLD H. KUEHN

For the past several years the writer has been engaged in an investigation of the available species of the Gymnoascaceae with respect to morphological and cultural characteristics. This series of studies was directed toward the establishment of sounder bases for taxonomy through the application of developmental morphological characters (**1955a**). As Benjamin recently pointed out (**1956**), only a detailed analysis of a large series of strains of species will bring order out of the confusion which exists now in several of the genera. At least one of them now consists of a heterogeneous assortment of species and it is possible that it, and some of the others, may be divided into several more consistent genera when more information has been accumulated. Moreover, the limits of the Gymnoascaceae and the relation of the members of this family to one another are uncertain.

The historical treatment of the Gymnoascaceae and of the fungi now included in this family has been presented in part in recent papers. The reader is referred to earlier papers by the writer (Kuehn, **1955a**, **1955b**, **1956**, **1957a**, **1957b**) and Benjamin (**1956**) for that information. Benjamin did not treat the species of the family, but only sought to establish generic distinctions. In this present series of two papers the writer hopes to clear up some of the confusion among the species. Preparation of a satisfactory monograph of the Gymnoascaceae must await the accumulation of additional detailed information on the structure and development of many more representatives of this family.

Baranetzky (**1872**) was the first to apply the name Gymnoascaceae to the group, a natural one to him, of Ascomycetes which lacked true perithecia (*Exoascus, Taphrina, Endomyces, Saccharomyces, Gymnoascus*). Eidam (**1880**) accepted this interpretation and further distinguished parasitic Gymnoascaceae (*Ascomyces, Exoascus, Taphrina*) from saprophytic members (*Ascodesmis, Ctenomyces, Gymnoascus*). Eidam remarked that on account of their developmental characters *Gymnoascus* and *Ctenomyces* differed from the other genera and formed a natural group within the family. Winter (**1887**) sanctioned this view, which was adopted by German mycologists. He divided the Ascomy-

cetes into four orders, Gymnoasceae, Pyrenomycetes, Hysteriaceae and Discomycetes. Brefeld (**1891**) divided the Ascomycetes into two subclasses, the Exoasci and the Carpoasci, with the Gymnoasci (with two genera, *Gymnoascus* and *Ctenomyces*) the first of five orders in the Carpoasci. Schroeter (**1893**) established the present limits of the Gymnoascaceae when he limited the family to saprophytic organisms with a non-membranous, reticulate peridium. He included five genera in the family, *Myxotrichum* Kunze (**1823**), with *M. chartarum* and *M. uncinatum*, *Arachniotus* Schroeter (**1893**), with *A. candidus*, *A. aureus*, and *A. ruber*, *Gymnoascus* Baranetzky (**1872**), with *G. reessii* and *G. setosus*, *Amauroascus* Schroeter (**1893**), with *A. niger* and *A. verrucosus*, and *Ctenomyces* Eidam (**1880**), with *C. serratus*. However, Massee (**1895**) and Matruchot and Dassonville (**1899a**) subsequently proposed other arrangements of the Gymnoascaceae. Massee regarded the family as being characterized by the complete absence of a peridium. He erected the sub-family Gymnoascaceae to include *Gymnoascus* and *Ascodesmis*, and the sub-family Ascomyceae to include *Ascomyces* and *Taphrina*. In so doing he indicated his agreement with Winter's interpretation of the family. Matruchot and Dassonville, contrary to Winter, followed the ideas of Schroeter and Brefeld in considering the Exoasci and Gymnoasci to be natural groups not related to each other. However, since certain species of Gymnoascaceae appeared to them to have a suggestion of a stalk as in *Onygena*, Matruchot and Dassonville divided the family into 3 tribes as follows: 1. Endomycétées—*Endomyces*; 2. Onygénées—*Onygena*; 3. Gymnoascées—*Gymnoascus*, *Ctenomyces*, *Trichophyton*, *Achorion* and *Microsporum*.

The relation of the dermatophytes to the Gymnoascaceae is discussed in many papers which have appeared since the publication of the paper in which Matruchot and Dassonville included certain dermatophytes in this family. Much of the evidence in support of this idea could not be duplicated by subsequent investigators. The reader is referred to several of the more important papers in which the problem is discussed more completely (Tate, **1992**; Chalmers and Marshall, **1914**; Ota and Langeron, **1923**; Pollacci, **1925**, **1932**; Nannizzi, **1926**, **1927**) and to a series of papers by Wilenczyk in which he describes asci produced by various species of dermatophytes (**1927**, **1928a**, **1928b**, **1928c**, **1929a**, **1929b**, **1929c**).

Clements and Shear (**1931**) included in this family several genera not now considered to belong here. Benjamin (**1956**) discussed the disposition of these excluded genera and the reader is referred to his paper. The following ten genera were placed by Benjamin in the

Gymnoascaceae: *Byssochlamys, Arachniotus, Amauroascus, Pseudogymnoascus, Gymnoascus, Eidamella, Arthroderma, Shanorella, Myxotrichum* and *Ctenomyces.* Since the appearance of Benjamin's paper the genus *Pseudoarachniotus* has been added (Kuehn, **1957b**). The following key shows the relationship existing between *Pseudoarachniotus* and the other genera in the family. Each of the genera and the included species will be discussed with the exception that treatment of *Gymnoascus* and *Myxotrichum* is reserved for a second paper in this series.

GYMNOASCACEAE Baranetzky, Bot. Zeit. **30**: 158. 1872.

Ascocarp a cleistothecium, or a peridium may be absent. Peridial hyphae loosely intertwined, non-membranous, thin or thick-walled, smooth-walled, echinulate or tuberculate, with or without appendages of various types. Appendages may be coiled spirally, branched comb-like, or long, simple, and either straight or uncinate. Asci irregularly crowded in the ascocarp, usually formed through the intervention of croziers, globose to sub-globose, or clavate, 8-spored usually, evanescent. Ascospores unicellular, globose, oblate, ovoid, or lenticular, smooth or variously sculptured. Imperfect phase represented by oidia, chlamydospores or conidia.

TYPE GENUS: *Gymnoascus* Baranetzky.

KEY TO THE GENERA OF THE FAMILY GYMNOASCACEAE
(adapted from Benjamin)

A. Peridial hyphae thin-walled, similar to vegetative hyphae, or absent.....B
AA. Peridial hyphae thick-walled, conspicuously different from vegetative hyphae.....E
B. Peridial hyphae absent.....C
BB. Peridial hyphae present, thin-walled.....D
C. Imperfect stage represented by conidia borne on phialides of a *Paecilomyces* type.....*Byssochlamys*
CC. Imperfect stage present or absent, never represented by conidia borne on phialides.....*Pseudoarachniotus*
D. Ascospores colorless or light-colored.....*Arachniotus*
DD. Ascospores brown or brown-violet.....*Amauroascus*
E. Peridial hyphae uniform, without spine-like branches or other appendages.....*Pseudogymnoascus*
EE. Peridial hyphae bearing spine-like branches or other appendages.....F
F. Appendages not conspicuously different from peridial hyphae; consisting of short spines or blunt projections.....G
FF. Appendages well-defined; often unlike peridial hyphae.....H
G. Peridial hyphae anastomosed; forming a well-defined net.....*Gymnoascus*

GG. Peridial hyphae not anastomosed; forming a bramble-like aggregate; peridial hyphae bearing deflexed spine-like branches; certain internal hyphae terminating in short, hyaline coils.....*Eidamella*
H. Appendages at least in part consisting of slender, elongate hyphae forming tight spirals.....I
HH. Appendages not in the form of spirals.....J
I. Peridial hyphae composed of uniform, asperulate, dumb-bell-shaped cells; spiral appendages few.....*Arthroderma*
II. Peridial hyphae disarticulating and composed of cells very irregular in size and shape; spiral appendages many.....*Shanorella*
J. Appendages elongate, simple, straight, curved or uncinate..*Myxotrichum*
JJ. Appendages ctenoid, several-celled with each cell straight or curved, possessing a recurved tooth-like outgrowth.....*Ctenomyces*

BYSSOCHLAMYS Westling, Svensk Bot. Tidskr. **3**: 134. 1909.

Discrete cleistothecia absent, each cluster of asci completely lacking a peridium. Asci conglomerate, globose, 8-spored. Ascospores ovate, smooth, hyaline. Imperfect phase represented by conidia borne on phialides of a *Paecilomyces* type; chlamydospores present or absent.

TYPE SPECIES: *Byssochlamys nivea* Westling.

KEY TO THE SPECIES OF BYSSOCHLAMYS

A. Chlamydospores present; conidia ovate to ellipsoidal; ascospores 2.8–4.0 × 3.5–5.6 μ.....1. *B. nivea*
AA. Chlamydospores absent; conidia ovate to fusiform; ascospores 4.3–4.5 × 6–6.5 μ.....2. *B. fulva*

1. B. NIVEA Westling, Svensk Bot. Tidskr. **3**: 125. 1909.
Arachniotus trisporus Hotson, Mycologia **28**: 500. 1936.
Gymnoascus sudans Vailionis, Didz. Univ. Mat. Gamt. **11**: 115. 1936.
Byssochlamys trisporus Cain, Can. Jour. Bot. **34**: 140. 1956.

Cleistothecia absent; asci in clusters with or without loosely aggregated white vegetative hyphae about them. Ascus groups more or less globose, mostly 37–151 μ diam., although individual clusters may be confluent. Asci hyaline, obovate, ranging in various strains from 5–12 × 6–16 μ, but mostly 7–9 × 10–13 μ, wall evanescent, 8-spored. Ascospores hyaline, ovoid to elliptical, smooth, 2.8–4.0 × 3.4–5.6 μ. Conidia borne in chains on phialides of a *Paecilomyces* type, hyaline, one-celled, ellipsoid to ovate, varying from 1.5–4.5 × 2.2–6.5 μ, but mostly 2.0–4.5 × 3.0–5.5 μ. Chlamydospores borne single, usually terminal, rarely intercalary, ovoid to pyriform, thick-walled, hyaline to buff, smooth or slightly roughened, measuring from 4–10 × 5–12 μ, but mostly 6.1–8 ×

7–11 μ. Vegetative hyphae pure white, usually becoming slightly yellow or cream color with age, consisting of thin hyphae 1.2–1.5 μ diam. and stouter hyphae measuring 4.2–8.4 μ diam. Racquet mycelium is also present. Small colorless droplets of exudate later collect to form large yellow drops.

TYPE LOCALITY: Sweden.

HABITAT: On botanical specimens preserved in ethanol, in soil, coal, cow's milk and in nutrient solution in which birch (*Betula verrucosa*) twigs were cultivated.

DISTRIBUTION: Lithuania, Sweden, and widely in the United States.

ILLUSTRATIONS: Westling, Svensk Bot. Tidskr. **3**, *pl. 4;* Vailionis, Didz. Univ. Mat. Gamt. **11**, *pl. 1–2;* Hotson, Mycologia **28**, *f. 1–13;* Rosenbaum, Ann. Missouri Bot. Gard. **31**, *pl. 1–6;* Benjamin, Aliso **3**, *pl. 1, f. 1–5;* Kuehn, Mycologia **49**, *f. 19–30, 32;* Gäumann, Pilze, *f. 102,1–103.*

The above description is a composite of those given by Westling (**1909**), Hotson (**1936**), Rosenbaum (**1944**), Vailionis (**1936**) and Kuehn (**1957**). This species seems to be quite variable and several distinct strains exist. However, there is no doubt that they all represent the same species. A more complete description of *B. nivea* including cultural characteristics and developmental morphology is presented by Kuehn (**1957a**), while Rosenbaum (**1944**) discusses the nuclear cytology in detail. For a discussion of the synonomy of this species see Benjamin (**1956**) and Kuehn (**1975a**). Karow and Foster (**1944**) reported clavacin production by a *Gymnoascus* sp. No. 5070.1 of the Thom collection. This specimen, also designated as NRRL 1678, was identified by the writer (**1954**) as *Arachniotus trisporus,* which since then was recognized to be synonomous with *B. nivea.*

2. B. FULVA Olliver and Smith, Jour. Bot. **71**: 197. 1933.

Cleistothecia absent, the asci in roughly globose clusters without any trace of a peridium. Asci hyaline, globose, 8-spored, measuring 11–12 μ diam. Ascospores hyaline, smooth, ovate, with dimensions of 4.3–4.5 × 6–6.5 μ. Conidial fructification of the *Paecilomyces* type, conidia hyaline, one-celled, ovate to elongate or fusiform, variable in size, mostly 2.3–2.5 × 4–9 μ, borne in long chains. Colonies white, then buff to pale-brown, reverse pale-brown.

TYPE LOCALITY: England.

HABITAT: Canned and bottled fruits, soil.

DISTRIBUTION: England, United States, Australia.

ILLUSTRATIONS: Olliver and Smith, Jour. Bot. **71**, *pl. 602, f. 1–2;* Raper and Thom, Man. Penicillia, p. 690, *f. 171;* Benjamin, Aliso **3**, *pl. I, f. 6.*

This description is taken from Olliver and Smith (**1933**) and Raper and Thom (**1949**). Although ascosporic strains appear to be infrequent in the United States, they occur commonly in England where they are important agents in the spoilage of canned fruit. In their original description Olliver and Smith stated that the conidial structure is very similar to *Paecilomyces varioti* Bainier. Raper and Thom maintain that there is little doubt that *P. varioti* and *B. fulva* are stages in the life cycle of the same fungus. Strains of *P. varioti* have been isolated from soil in all parts of the world, and recently *B. fulva* was isolated from soil in Australia (Thower, **1954**). Emmons (**1935**) discussed the development of the gametangial initials, while Olliver and Smith and Raper and Thom presented the most complete discussions to date of the cultural and morphological characteristics of this species.

AMAUROASCUS Schroeter, Cohn Krypt.-Fl. Schles. **3** (*2*): 211. 1893.

Cleistothecia globose. Peridial hyphae thin-walled, uniform, loosely interwoven. Asci 8-spored. Ascospores globose or ellipsoid, echinulate, brown or brown-violet.

TYPE SPECIES: *Amauroascus niger* Schroeter.

KEY TO THE SPECIES OF AMAUROASCUS

A. Black ascospore mass; ascospores ellipsoid, 3.5–4.0 × 4.5–6 μ.....1. *A. niger*
AA. Ascospore mass chestnut-brown; ascospores globose, 8.5–10 μ diam.
.....2. *A. verrucosus*

1. A. NIGER Schroeter, Cohn Krypt.-Fl. Schles. **3** (*2*): 211. 1893.

Cleistothecia white, globose or ellipsoid, 0.5–1.5 mm diam. Peridial hyphae hyaline, thin-walled. Asci ellipsoid, 10–12 × 11–14 μ, 8-spored, wall evanescent. Ascospores brown, black in mass, delicately echinulate, ellipsoid, 3.5–4 × 4.5–6 μ. Imperfect stage unknown.

TYPE LOCALITY: Germany.

HABITAT: Old badger dung, soil.

DISTRIBUTION: Germany; Ssrednjaja Rogatka, U.S.S.R.

ILLUSTRATIONS: Gilman, Man. Soil Fungi, p. 166, *f. 42.*

The above description is adapted from those of Schroeter (**1893**) and Gilman (**1957**).

2. A. VERRUCOSUS (Eidam) Schroeter, Cohn Krypt.-Fl. Schles. **3** (2): 211. 1893.

Gymnoascus verrucosus Eidam, Ber. Bot. Sect. Schles. Ges. **1886**: 162. 1886.

Cleistothecia spherical, at first white but turning brown as the ascospores mature, 3–4 mm diam. Peridium very thin, composed of hyaline, thin-walled hyphae. Asci in chains, pyriform, 8-spored, 16–20 μ diam. Ascospores globose, brown, in mass chestnut-brown, echinulate, 8.5–10 μ diam. Asexual phase unknown.

TYPE LOCALITY: Germany.

HABITAT: Rotting shoe leather.

DISTRIBUTION: Germany, France.

ILLUSTRATIONS: Dangeard, Botaniste **10**, *pl. 8–10;* Dodge, Med. Mycol. p. 426, *f. 71;* Gäumann, Pilze, *f. 104.*

The description of this species is based on those of Eidam (**1886**), Schroeter (**1893**) and Dangeard (**1907**). The writer has not seen any material representing this genus. Aside from the original descriptions, only Dangeard (**1907**), Raillo (**1928, 1929**) and Guillermond (**1903**) have published reports of *Amauroascus.* The distinguishing characteristic between *Arachniotus* and *Amauroascus* as they are defined by Schroeter is in the color of the ascospores, hyaline or bright colored in the former genus, shades of brown in the latter. As Benjamin has recently indicated (**1956**), species in these two genera may well be congeneric, but such a re-evaluation must await the re-isolation of the species of *Amauroascus.*

ARACHNIOTUS Schroeter, Cohn Krypt.-Fl. Schles. **3** (2): 210. 1893.

Cleistothecia more or less globose, white or light-colored. Peridial hyphae thin-walled, uniform in diameter and appearance, loosely interwoven. Asci 8-spored, wall evanescent. Ascospores spherical, ovoid, oblate, or ellipsoid, hyaline or light-colored, smooth or sculptured.

TYPE SPECIES: *Arachniotus candidus* (Eidam) Schroeter.

KEY TO THE SPECIES OF ARACHNIOTUS

A. Ascocarps not white at maturity ..... B
AA. Ascocarps white ..... E
  B. Ascocarps usually brown at maturity; ascospores globose, reticulate, hyaline ..... 1. *A. reticulatus*
  BB. Ascocarps not in shades of brown; ascospores not reticulate, not hyaline, usually not globose C

C. Ascocarps golden-yellow, with fine hyphal spirals in the peridium; ascospores golden-yellow, echinulate.....2. *A. aureus*
CC. Ascocarps in shades of red; peridial spirals absent; ascospores not in shades of yellow.....D
D. Ascocarps brick-red at maturity; ascospores smooth, subglobose to ellipsoid, red to orange-red.....3. *A. ruber*
DD. Ascocarps rust-red to orange-red at maturity; ascospores lenticular, rust-red, smooth.....4. *A. dankaliensis*
E. Ascocarps large, over 0.5 mm; asci small, 5–8 μ diam.; ascospores below 4 μ diam. .....F
EE. Ascocarps small, 135–250 μ diam.; asci large, 13.5 μ diam.; ascospores 3–4 × 5.4–6 μ.....5. *A. terrestris*
F. Ascospores smooth, ellipsoid, 2.5 × 3–3.5 μ; asci 5–6 μ diam. .....6. *A. candidus*
FF. Ascospores echinulate, ovoid to elliptical, 2–2.5 × 3.2–4 μ; asci 7–8 μ diam.....7. *A. trachyspermus*

1. A. RETICULATUS Kuehn, Mycologia **49**: 57. 1957.

Cleistothecia roughly globose, yellow on PDA, light-brown on Sabouraud's agar, 126–883 μ diam. Peridial hyphae smooth or tuberculate, thin-walled, light-yellow with segments tinged brown. Asci hyaline, oblong to clavate, 7–8.4 × 11.2–12.6 μ, 8-spored, wall evanescent. Ascospores globose, hyaline, reticulate, with thick, elevated bands, measuring 2.8–3.2 μ with the bands, 2.6–3 μ excluding the bands. Vegetative hyphae hyaline, 0.8–2.1 μ diam. Racquet mycelium also present. Oidia hyaline, 2–2.8 × 2.8–8.4 μ. Distinctive, more or less earthy, odor is present.

TYPE LOCALITY: Georgia.
HABITAT: Bat dung.
DISTRIBUTION: Known only from the type collection.
ILLUSTRATIONS: Kuehn, Mycologia **49**, *f. 1–18, 31.*
This description is taken from the original.

2. A. AUREUS (Eidam) Schroeter, Cohn Krypt.-Fl. Schles. **3** (*2*): 210. 1893.
Gymnoascus aureus Eidam, Ber. Bot. Sect. Schles. Ges. **1886**: 161. 1886.

Ascocarp round, 0.5–1 mm diam., golden-yellow. Peridial hyphae loosely interwoven, golden-yellow, thin-walled, slender, ending in delicate, thin spirals. Asci 8-spored. Ascospores golden-yellow, globose to elliptical, echinulate, 3.5–4 μ diam. Conidial phase absent.

TYPE LOCALITY: Germany.
HABITAT: Bread, paper, rotting vegetables.

DISTRIBUTION: Apparently only from the type locality.

ILLUSTRATION: Nannizzi, Ann. Myc. **24**, *f. 10*.

This description is adapted from those of Eidam (**1886**), Schroeter (**1893**), and Rosenbaum (**1944**). Several isolates labeled *A. aureus* have been received by the writer. However, none of these can be placed in this species with assurance, and we must await its re-isolation before any definite generic assignment can be accorded *A. aureus*.

3. A. RUBER (Van Tieghem) Schroeter, Cohn Krypt.-Fl. Schles. **3** (*2*): 211. 1893.

Gymnoascus ruber Van Tieghem, Bull. Soc. Bot. Fr. **24**: 159. 1877.

Ascocarp more or less round, about 0.5 mm diam., often confluent, pale-yellow first, then orange, becoming dark red-orange or brick-red. Peridial hyphae loosely interwoven, thin-walled, uniform, possessing red granules. Asci round, 8-spored, wall evanescent, 10–12 μ diam., often very few (6–20) per ascocarp. Ascospores subglobose to ellipsoid, orange-red or red, smooth with an equatorial furrow, 3.5–4.5 × 4.5–5.5 μ. Conidial phase absent according to Eidam (**1886**), but present and *Verticillium*-like according to Van Tieghem.

TYPE LOCALITY: France.

HABITAT: Dog, rat, cat and goat dung.

DISTRIBUTION: Germany, France, England, Wales, Gold Coast.

ILLUSTRATIONS: Drawings of Massee now located in the herbarium of the New York Botanical Garden.

This description is based on those of Van Tieghem (**1877**), Eidam (**1886**), Schroeter (**1893**), Smith (**1896**), Hotson (**1936**), Rosenbaum (**1944**) and Massee (**1895**). Nothing resembling this species has ever been examined by the writer and the ultimate generic placement of *A. ruber* must await its re-isolation.

4. A DANKALIENSIS (A. Cast.) Beyma, Ant. van Leeuwenhoek Jour. Microbiol. Serol. **8**: 107. 1942.

Trichophyton dankaliense Aldo Castellani, Jour. Trop. Med. Hyg. **40**: 315. 1937.

Mycelium felt-like, yellow at first, later brownish-yellow to rust-red or orange-red. Ascocarps round, variable in size. Peridial hyphae thin-walled, uniform, light-brown to rust-brown, with round enlargements scattered on the hyphae; peridial hyphae also forming round, brown "Hulle" cells, 10–12 μ diam. Asci short pedicellate, 11–13 μ diam., 4-, 6- or 8-spored. Ascospores rust-red, smooth, lenticular, with an equatorial bulge, 4.3–5.3 × 6–8 μ. No asexual stage known.

TYPE LOCALITY: Rome, Italy.

HABITAT: Hairy skin of a camel.

DISTRIBUTION: Known only from the type collection.

ILLUSTRATIONS: Beyma, Ant. van Leeuwenhoek Jour. Microbiol. Serol. **8**: 106, *f. 1, a–f;* Castellani, Jour. Trop. Med. Hyg. **40**, *pl. 1–3.*

The above description is taken from that of Beyma (**1942**). A transfer of the type strain is maintained in the culture collection of the Centraalbureau voor Schimmelcultures. Through the courtesy of Dr. Libero Ajello, Communicable Disease Center, the writer has obtained a sub-culture of the type strain, but has not had occasion to examine it. Superficial examination proves Beyma to be correct in her description of the mycelial color characteristics. Moreover, on Sabouraud's maltose agar this species produces an extremely disagreeable and characteristic odor.

5. A. TERRESTRIS Raillo, Zentral. Bakt. Parasit. Inf. Abt. *2*, **78**: 521. 1929.

Cleistothecia round, 135–250 μ diam. Peridial hyphae white, delicately interwoven. Asci globose or oval, 13.5 μ diam., 8-spored. Ascospores hyaline, ovoid, 3–4 × 5.4–6 μ.

TYPE LOCALITY: Ssrednjaja Rogatka, U.S.S.R.

HABITAT: Soil.

DISTRIBUTION: Known only from the type collection.

ILLUSTRATIONS: Raillo, Zentral. Bakt. Parasit. Inf. Abt. *2*, **78**: 521, *f. 3;* Gilman, Man. Soil Fungi, p. 166, *f. 41.*

The above description is adapted from the original. This species is not to be found at present among the culture collections of the world. It must be re-isolated before any satisfactory disposition can be achieved.

6. A. CANDIDUS (Eidam) Schroeter, Cohn Krypt.-Fl. Schles. **3** (*2*): 210. 1893.

Gymnoascus candidus Eidam, Ber. Bot. Sect. Schles. Ges. **1886**: 161. 1886.

Fruiting bodies snow-white, and remaining so or becoming yellow-white with age, globose, often confluent, 0.5–2 mm diam.; peridial hyphae (absent according to Dale, **1903**) irregularly branched, scanty, thin-walled, smooth and delicate. Asci ovoid to globose, 8-spored, wall evanescent, 5–6 μ diam. Ascospores hyaline, ellipsoid, smooth, 2.5 × 3–3.5 μ. Asexual phase represented by oidia and pyriform chlamydospores in chains with sterile intercalary segments.

TYPE LOCALITY: Germany.

HABITAT: Cooked rice, bees nest, owl nests, owl feathers, and dung of roe, fox and owl.

DISTRIBUTION: Germany, England.

ILLUSTRATIONS: Massee and Salmon, Ann. Bot. **16**, *pl. 4, f. 11–12;* Dale, Ann. Bot. **17**, *f. 40–60.*

The foregoing description was compiled from the literature of Eidam (**1886**), Schroeter (**1893**), Massee and Salmon (**1902**), and Dale (**1903**).

7. A. TRACHYSPERMUS Shear, Science II **16**: 138. 1902.

Ascocarp globular, up to 0.5 mm diam., white. Peridial hyphae thin-walled, uniform, loosely interwoven. Asci globose to subglobose, 7–8 μ diam., 8-spored. Ascospores almost hyaline, in mass greenish-yellow according to Rosenbaum (**1944**), and light lemon-yellow according to Shear (**1931**), echinulate, ovoid to elliptical, 2–2.5 × 3.2–4 μ. Conidial apparatus resembles *Penicillium;* chlamydospores absent according to Hotson's interpretation (**1936**).

TYPE LOCALITY: Jamesburg, New Jersey.

HABITAT: Diseased cranberries (*Vaccinium macrocarpon*).

DISTRIBUTION: New Jersey.

ILLUSTRATIONS: Shear, USDA Bur. Pl. Ind. Bul. **110**, *pl. 4, f. 18–22;* Shear *et al.*, USDA Tech. Bul. **258**: 13, *f. 14.*

This description is taken from those of Shear (**1902, 1907**), Hotson (**1936**) and Rosenbaum (**1944**). Until the species is re-isolated and studied carefully no final conclusion can be attained concerning its generic affiliations. Not only could it be a representative of *Arachniotus, Pseudoarachniotus* or *Byssochlamys,* but it also might belong in *Talaromyces.*

Through the courtesy of Mr. John A. Stevenson, the writer examined the 10 specimens of this species on deposit in the National Fungus Collections. Two of these specimens were labeled as type material, and the other 8 were also isolated and identified by C. L. Shear, and formed part of his original collections. Nine of the specimens showed the characteristics of this species as described by Shear, while the tenth specimen definitely did not represent *Arachniotus trachyspermus.*

These specimens represented several strains, a fact which was evident from the varying length of the ascospore echinulations among the specimens. Two of the strains had long echinulations, as figured by Shear (**1931**), while others had much shorter echinulations.

Shear made no mention of chlamydospores, but in one of his specimens the writer found numerous chlamydospores. These were spherical, 5.5–9.9 μ diam., hyaline, smooth, thick-walled and borne singly, both lateral and intercalary. In this same specimen, there were free conidia present, but no conidial apparatus could be located. The conidiospores were smooth, globose, green, and 2.5–2.8 μ diam.

Ascospore size varied slightly from one specimen to another. In three specimens the measurements were as follows: 2.2–3 × 3.3–3.5 μ, 2.5 × 3–4 μ, and 2.2 × 3.3 μ. All specimens had hyaline ascospores. The color of the ascocarps in all the specimens varied from very pale yellow to creamy white.

It can be seen from this that *Arachniotus trachyspermus* shows certain affinities to the *Talaromyces* group. These are: 1. light yellow to cream colored fruit body; 2. elliptical ascospores; 3. echinulate or spinulose ascospores; 4. penicillate conidial apparatus; 5. conidial phase infrequently present; 6. habitat on cranberries (*Penicillium striatum* was first isolated from canned blueberries).

A study of the members of the *Talaromyces* series was made to see where *A. trachyspermus* might be placed. It is similar to *Talaromyces* in the loosely interwoven peridial hyphae and in particular it is quite like *T. spiculisporus* in the nature of the ascocarp as well as the ascospores. The ascospores of both are not only echinulate and elliptical, but those of *T. spiculisporus* measure 2.2–2.8 × 3–3.5 μ while those of *A. trachyspermus* (according to the measurements of the writer on Shear's specimens) are 2.2–3.0 × 3.3–3.5 μ.

PSEUDOARACHNIOTUS Kuehn, Mycologia **49**: 694. 1957.

Discrete cleistothecia lacking; peridial hyphae absent. Ascocarp initials represented by discrete ascogonia and antheridia. Asci 8-spored, formed by means of croziers, wall evanescent. Ascospores hyaline or shades of red or yellow, globose, ovoid, elliptical or lenticular, smooth or sculptured. Imperfect stage present or absent, never represented by conidia borne on phialides. Racquet mycelium present or absent.

TYPE SPECIES: *Pseudoarachniotus roseus* Kuehn.

KEY TO THE SPECIES OF PSEUDOARACHNIOTUS

A. Colonies red or shades of red-orange; ascospores red, tinged orange-yellow ..... 1. *P. roseus*
AA. Colonies in shades of yellow; ascospores lemon-yellow ..... 2. *P. citrinus*

1. P. ROSEUS Kuehn, Mycologia **49**: 695. 1957.

Cleistothecia lacking. Asci occurring in exposed groups scattered among the vegetative hyphae; groups of asci 22–45 μ diam., but often confluent into larger clusters. Asci hyaline, ovoid to obovate, 8.4–9.8 × 8.8–12.6 μ, wall evanescent, 8-spored. Ascospores red, tinged orange-yellow, ovoid to lenticular, 3.6–5.6 × 4–7 μ, often slightly angular, otherwise smooth. Vegetative hyphae hyaline, 1.2–3.2 μ diam.; racquet mycelium infrequently present. Asexual stage unknown.

TYPE LOCALITY: Tucson, Arizona.

HABITAT: Ulcerated gizzard of a chicken.

DISTRIBUTION: Known only from the type locality.

ILLUSTRATIONS: Kuehn, Mycologia **49**, *f. 1–18, 30.*

The above description is adapted from the original. Three specimens of this species are located in the herbarium of the National Fungus Collections, Beltsville, Maryland. They are identified as Thom culture No. 5439, and were isolated in Arizona in 1934–1935.

2. P. CITRINUS (Massee and Salmon) Kuehn, Mycologia **49**: 699. 1957.
Arachniotus citrinus Massee and Salmon, Ann. Bot. **16**: 62. 1902.

Cleistothecia absent. Clusters of asci exposed and scattered among the vegetative hyphae; groups of asci variable in size, often confluent. Asci ovoid, hyaline, 8.8–9.3 × 10.8–12.8 μ, 8-spored, wall evanescent. Ascospores smooth, lemon-yellow, subglobose or elliptical; subglobose spores 4.8–5.1 μ diam., and elliptical spores 3.1–4.4 × 5.3–6 μ, slightly compressed, wall thickened terminally and in a median band. Vegetative hyphae lemon-yellow, 1.2–3 μ diam. Racquet mycelium absent. Imperfect spores unknown.

TYPE LOCALITY: Kew, England.

HABITAT: Kangaroo dung, soil.

DISTRIBUTION: England, Tennessee.

ILLUSTRATIONS: Massee and Salmon, Ann. Bot. **16**, *pl. 5, f. 86–88;* West and Ajello, Mycologia **48**: 164, *f. 1, A–D;* Kuehn, Mycologia **49**: 698, *f. 19–29.*

The above description is taken from that presented by Kuehn (**1957b**). Massee and Salmon followed the precedent established by Schroeter in considering the vegetative hyphae, which are located in close proximity to the groups of asci, to constitute a peridium. Although West and Ajello (**1956**) noted the complete absence of a peridium, they felt obliged to follow Schroeter's interpretation of the genus *Arachniotus*. However, as recently discussed by Kuehn (**1957b**), this

species does not conform to Schroeter's concept of the genus and should be transferred to *Pseudoarachniotus*.

The measurements of the Tennessee strain differ slightly from those cited by Massee and Salmon. The asci of the London strain measured 8–10 μ diam., and the ascospores were 2.5–3.2 × 4–5 μ.

ARTHRODERMA Berkeley, Outl. Brit. Fungol. 357. 1860.

Cleistothecia white to pale-yellow, globose. Peridial hyphae hyaline, branched and anastomosed, thick-walled, densely asperulate, with constricted, symmetrical or asymmetrical, dumbbell-shaped cells; thin-walled, septate, elongate, spiral appendages occurring terminally or laterally, with numerous free ends. Asci globose, 8-spored, wall evanescent. Ascospores yellow in mass, ovoid, smooth. Chlamydospores (aleuriospores) abundant, clavate.

TYPE SPECIES: *Arthroderma curreyi* Berkeley.

1. A. CURREYI Berkeley, Outl. Brit. Fungol. 357. 1860.
Illosporium curreyi (Berk.) Saccardo, Syll. Fung. **4**: 660. 1886.
Ctenomyces xylophilus Höhnel, Ann. Myc. **3**: 416. 1905.

Cleistothecial characteristics are those of the genus. Asci globose to subglobose, 4–5 × 5 μ, 8-spored, wall evanescent. Ascospores hyaline, yellow in mass, smooth with a slight equatorial groove, small, ovoid, 2 × 2.4–3.3 μ. Asexual phase represented by clavate chlamydospores, intercalary and terminal.

TYPE LOCALITY: England.

HABITAT: Dead leaves and sticks, wood, feathers, horn, dung.

DISTRIBUTION: England, France, Panama, Italy, Austria, Germany, Massachusetts, Maine, Illinois.

ILLUSTRATIONS: Benjamin, Aliso **3**, *pl. 3, 4;* Currey, Quart. Jour. Micr. Soc. **2**, *pl. 9, f. 6–8;* Cooke, Handb. Brit. Fungi **2**: 626; Nannizzi, Ann. Myc. **24**, *f. 5a, pl. 11, f. a–m;* Gäumann, Pilze, *f. 105;* Fischer, E. & P. Pflanzenf. **1** (1), *f. 211 A–F;* Dangeard, Botaniste **10**, *pl. 6–7;* Winter, Krypt.-Fl. Deuts. **2**: 13; Dodge, Med. Mycol., p. 427, *f. 73, 3, f. 74, 1;* Smith, Jour. Bot. **41**, *pl. 454, f. 1–2;* Eidam, Cohn Beitr. Biol. Pfl. **3**, *pl. 12, f. 1, 3–8, pl. 13, f. 9–24, pl. 14, f. 27–33, pl. 15, f. 35–38;* Salmon, Jour. Queckett Micr. Club **7**, *pl. 20, f. 11–13.*

The above description is taken from those of Benjamin (**1956**) and Eidam (**1880**). The descriptions given by Berkeley (**1860**) and Currey (**1854**) are fragmentary; however, the figures of this distinctive fungus presented by Currey are adequate to permit definite recognition of the organism with which he worked. According to Benjamin (**1956**) *A.*

curreyi has been mistaken for the perfect phase of *Ctenomyces serratus* Eidam. Eidam in reporting *Ctenomyces* had before him a perfect and an imperfect stage, or so he thought, representing the same fungus. However, the imperfect stage which Eidam had is actually the perfect stage of *C. serratus,* while the perfect stage he had represents *A. curreyi.* Therefore, all the succeeding investigations of the perfect stage of *C. serratus* in reality discuss *A. curreyi,* and the development of the perfect stage of *C. serratus* has never been investigated. Saccardo (**1886**) placed this species in *Illosporium* but Salmon (**1900**) refuted this proposal. Höhnel (in Rehm, **1905**) isolated *A. curreyi* and at first described it as a new species, *Ctenomyces xylophilus,* on the basis that *C. serratus* is the same as *A. curreyi* according to Smith and Rea (**1902b**), and Höhnel realized that his isolate was not *C. serratus.* However, later he recognized that his isolate was *A. curreyi,* and that *A. curreyi* and *C. serratus* are not the same (Rehm, **1905**).

CTENOMYCES Eidam, Cohn Beitr. Biol. Pfl. **3**: 274. 1880.

Cleistothecia orange-brown, globose. Peridium of densely interwoven, slender, pale-orange, thin-walled hyphae surrounded by a network of anastomosed, thick-walled, septate, asperulate, orange-brown hyphae from which arise many curved, septate, ctenoid appendages. Appendages consisting of about 5–11 thick-walled asperulate cells; each cell, excluding the elongate basal one or two cells, growing outward distally on one side to form a recurved, acuminate, tooth-like protuberance.

TYPE SPECIES: *Ctenomyces serratus* Eidam.

1. C. SERRATUS Eidam, Cohn Beitr. Biol. Pfl. **3**: 274. 1880.

Cleistothecial characteristics those of the genus; cleistothecia about 100–350 μ diam., excluding the appendages. Appendages 100–150 μ long, arising from thick-walled peridial hyphae 3.3–6 μ diam. Ascospores pale-orange in mass, slightly lenticular, more convex on one side, 2–2.6 $\times$ 3.3–3.6 μ.

TYPE LOCALITY: Germany.

HABITAT: Feathers, ass dung.

DISTRIBUTION: Germany, Liberia, Africa, Cuba, Panama, Italy, Massachusetts.

ILLUSTRATIONS: Benjamin, Aliso **3**, *pl. 5;* Gäumann, Pilze, *f. 102, 2–3;* Nannizzi, Ann. Myc. **24**, *f. 5, b;* Dodge, Med. Mycol., p. 428, *f. 74, 2–8;* Eidam, Cohn Beitr. Biol. Pfl. **3**, *pl. 12, f. 2.*

This description is adapted from the description of Benjamin (**1956**) compiled from material he examined in the Farlow Herbarium. Benjamin stated that this species represents what Eidam called the resting phase. In subsequent reports of *C. serratus* the organism at hand usually was not this species, but was *Arthroderma curreyi.* This aspect is discussed by Benjamin, and the reader is referred to his paper. Matruchot and Dassonville (**1899b**) also appear to have had *A. curreyi* since they report that the conidial phase of Eidam was absent, and they also speak of the spiral hyphal tips of the ascocarp. Should *C. serratus* be re-isolated a comparative study of the developmental morphology of *A. curreyi* and *C. serratus* should be undertaken and compared with the results reported by Eidam (**1880**) and Dangeard (**1907**).

EXCLUDED SPECIES

C. BOSSAE Milochevitch, Ann. Par. Hum. Comp. **13**: 566. 1935.

Milochevitch considered *Ctenomyces* and *Trichophyton* to be synonomous (**1935**). In describing this new species he stated that it is close to *Ctenomyces* (*Trichophyton*) *persicolor,* but is distinguished from it by producing a green, diffusible pigment, in being tardily pleomorphic, and in being severely pathogenic to guinea pigs. No asci were described, and illustrations show micro- and macroconidia, oidia and hyphae. This fungus was isolated from a case of sycosis in the beard of a student, 24 years of age, who had lived in Sarajevo for 2½ years prior to the appearance of the disease. This species was also isolated from hairs of a guinea pig. In culture the fungus is long-lived, flat but finely downy, and has the appearance of a deerskin.

C. XYLOPHILUS Höhnel, Ann. Myc. **3**: 416. 1905.

This is a synonym for *Arthroderma curreyi,* and a discussion of this synonomy is presented under the latter species.

PSEUDOGYMNOASCUS Raillo, Zentralbl. Bakt. Parasit. Inf. Abt. II **78**: 520. 1929.

Sterile mycelium white, diffuse. Cleistothecia globose, minute. Peridium formed of thick-walled, smooth, reticulate and copiously branched hyphae. Asci globose or slightly oval, 8-spored. Ascospores ovoid or globose. In structure similar to *Gymnoascus,* but differing in the absence of hooks and thorns in the peridium.

TYPE SPECIES: *Pseudogymnoascus vinaceus* Raillo.

KEY TO THE SPECIES OF PSEUDOGYMNOASCUS

A. Peridial hyphae yellow, of uniform diameter.....1. *P. vinaceus*
AA. Peridial hyphae red, thickened at the nodes.....2. *P. roseus*

1. P. VINACEUS Raillo, Zentralbl. Bakt. Parasit. Inf. Abt. II **78**: 520. 1929.

Cleistothecia globose, dark-red, 135–270 μ diam. Peridial hyphae golden-yellow, smooth, profusely branched, reticulate. Asci 5.4 μ diam., 8-spored. Ascospores hyaline, in mass red, globose or ovoid, 2 × 2.7–3 μ. Asexual-spore phase unknown.

TYPE LOCALITY: Chibiny and Ssrednajaja Rogatka, U.S.S.R.

HABITAT: Soil.

DISTRIBUTION: U.S.S.R., Barro Colorado Island (Canal Zone), Casita Alta (Panama), Colorado.

ILLUSTRATIONS: Raillo, Zentralbl. Bakt. Parasit. Inf. (Abt. II **78**: 520, *f. 1;* Gilman, Man. Soil Fungi, p. 167, *f. 43.*

The above description is based on that presented by Raillo (**1929**).

2. P. ROSEUS Raillo, Zentralbl. Bakt. Parasit. Inf. Abt. II **78**: 520. 1929.

Cleistothecia globose, red, 100–270 μ diam. Peridial hyphae red, smooth, richly-branched, reticulate, with characteristic thickenings at the nodes. Asci globose or oval, 5.4 μ diam., 8-spored. Ascospores hyaline, in mass red, globose or oval, 2 × 2.7–3 μ. No asexual phase known.

TYPE LOCALITY: Kamennaja Stepj, Gouvernement Woronesch, U.S.S.R.

HABITAT: Soil.

DISTRIBUTION: U.S.S.R., India, Iowa, Wisconsin.

ILLUSTRATIONS: Raillo, Zentralbl. Bakt. Parasit. Inf. Abt. II **78**: 520, *f. 2;* Taber, Proc. Iowa Acad. Sci. **58**, *pl. 2, f. 5.*

This description is taken from that of Raillo (**1929**). The writer has not examined material of this genus; however, through the courtesy of Drs. K. B. Raper and W. A. Taber he has obtained cultures of *P. roseus* for future study.

EIDAMELLA: Matruchot and Dassonville, Bull. Soc. Myc. Fr. **17**: 129. 1901.

Cleistothecia more or less globose, composed of a thorny aggregate of black, thick-walled, septate hyphae bearing numerous short, septate, apiculate, more or less deflexed branches with hyaline apices; many of the hyphae surrounding the central group of asci bear one or more short,

hyaline, spiral, terminal branches. Asci globose to ovoid, pedicellate, evanescent. Ascospores nearly hyaline, lenticular.

TYPE SPECIES: *Eidamella deflexa* (Berkeley) Benjamin.

1. E. DEFLEXA (Berkeley) Benjamin, Aliso **3**: 313. 1956.
   Myxotrichum deflexum Berkeley, Ann. Nat. Hist. **1**: 260. 1838.
   Eidamella spinosa Matr. and Dass., Bull. Soc. Myc. Fr. **17**: 123. 1901.
   Eidamella papyricola Saccas, Bull. Soc. Myc. Fr. **66**: 121. 1950.
   Myxotrichum ochraceum Berk. & Br. Ann. Mag. Nat. Hist. IV **15**: 37. 1875 (in part).

Cleistothecia spherical, black, 126–352 μ diam., including the appendages; ascocarps frequently much reduced, with 3 or 4 appendages surrounding a few asci. Peridial hyphae dark-brown, septate, branched, cuticularized, smooth. Appendages variable, up to 189 μ long 1.4–2.8 μ diam.; lateral branchlets straight, or usually deflexed, either upward or downward, 1.4–2 μ diam., up to 56 μ long, with hyaline apices. Shorter hyphae, located toward the interior among the asci, terminating in several hyaline spirals. Asci hyaline, obovate, clavate or ovoid, 4.2–7 $\times$ 12.6–25.2 μ, including the pedicels, 8-spored, wall evanescent. Ascospores hyaline, pale orange-yellow in mass, ovoid to elliptical-lenticular, delicately striate with minute longitudinal furrows, 2.4–2.9 $\times$ 3.6–5.2 μ. Racquet mycelium present. Asexual phase absent.

TYPE LOCALITY: France.

HABITAT: Ringworm lesions of a dog, diseased fingernail of a human, rotting rug, wood, paper, feathers, straw, wood shavings.

DISTRIBUTION: France, England, Argentina, Canada, Maine, Massachusetts, California.

ILLUSTRATIONS: Benjamin, Aliso **3**, *pl. 8;* DeLamater, Mycologia **29**: 572 et seq., *f. 1–27;* Matruchot and Dassonville, Bull. Soc. Myc. Fr. **17**, *pl. 5;* Berkeley, Ann. Nat. Hist. **1**, *pl. 8, f. 9;* Berkeley and Broome, Ann. Mag. Nat. Hist. IV **15**, *pl. 1, f. 4;* Massee and Salmon, Ann. Bot. **16**, *pl. 5, f. 80–82;* Matruchot and Dassonville, Rev. Mycol. **24**, *pl. 221, f. 8–15;* Saccas, Bull. Soc. Myc. Fr. **66**, *f. 1–4;* Ferro, Nuovo Giorn. Bot. Ital. II **14** (1907), *pl. 3, f. 3;* Peyronel, Ann. Myc. **12**: 467, *f. 3, 1.*

The above description is adapted from those of Kuehn (**1956**) and Benjamin (**1956**). For a discussion of the synonomy of this species the reader is referred to the papers of Kuehn, Benjamin, and Massee and Salmon (**1902**). The latter authors stated that *M. ochraceum* in part belongs here since the type specimen of *M. ochraceum* in Berkeley's herbarium contains only *M. deflexum* (now known as *E. deflexa*) and this was written on the packet already in an unknown hand. However,

other specimens of *M. ochraceum* sent out by Berkeley have proved to be *M. aeruginosum*. Ferro (**1907**) examined the *Myxotrichum* species in Saccardo's herbarium and came to the conclusion that *M. deflexum* represented a species of *Myxotrichella* Saccardo. DeLamater (**1937**) and Kuehn (**1956**) presented the morphological development of *E. deflexa*.

2. E. ACTONI Datta, Indian Vet. Jour. **8** (cited from Rev. Appl. Myc. **11**: 716. 1932. Also see Gregory, **1935**).

Cleistothecial characteristics those of the genus. This species differs from *E. deflexa* in possessing septate spines. Asci globose, many-spored. Chlamydospores intercalary, lateral and terminal; the intercalary and terminal chlamydospores globose, variable in size, the lateral chlamydospores less numerous, oval or pyriform, and either sessile or short-pedicellate. Conidial bushes also present.

TYPE LOCALITY: Calcutta, India.

HABITAT: Diseased skin of a dog.

DISTRIBUTION: Known only from the type collection.

ILLUSTRATIONS: Datta, Indian Vet. Jour. **8**, *pl. 1–2*.

The above description is taken from Gregory (**1935**) and Rev. Appl. Myc. (**1932**). Datta (**1932**) did not provide measurements to accompany his description, and merely indicated that his species differs from *E. deflexa* in possessing septate spines and multispored asci.

SHANORELLA Benjamin, Aliso **3**: 319. 1956.

Cleistothecia globoid, or ovoid, small, aggregated. Peridial hyphae thick-walled, septate, loosely reticulate, readily disarticulating at maturity; the cells more or less elongate, straight or curved, simple or branched, very irregular in size and shape, giving rise to coiled appendages composed of slender, elongate, septate hyphae. Asci 8-spored, evanescent. Ascospores yellow, oblate.

TYPE SPECIES: *Shanorella spirotricha* Benjamin.

1. S. SPIROTRICHA Benjamin, Aliso **3**: 319. 1956.

Cleistothecia globoid or ovoid, bright-yellow to golden-yellow, often confluent, small, about 100–200 μ diam., not including the coiled appendages. Peridial hyphae pale-yellow, septate, thick-walled, smooth, disarticulating at maturity; the cells more or less elongate, measuring about 3–15 $\times$ 10–35 μ, straight or curved, simple or branched, irregular in size and shape, the cell-wall 0.6–2 μ thick. Appendages numerous, consisting of slender (1–1.3 μ wide), thin-walled, hyaline, septate, uniform terminal or lateral outgrowths of peridial cells forming tight coils

of 20–30 turns; coils 10–20 × 25–40 μ. Asci hyaline, globoid, 5.2–7.8 μ diam., 8-spored, wall evanescent. Ascospores bright-yellow, flattened-oblate, 2 × 3.5–3.9 μ, smooth except for an elevated, equatorial band. Asexual phase represented by hyaline, terminal, lateral or intercalary, clavate or oblong, chlamydospores, measuring 2–3.3 × 3.3–12 μ.

TYPE LOCALITY: Claremont, California.

HABITAT: Feathers of a dead bird, chicken feathers, rabbit fur (all in moist chamber cultures).

DISTRIBUTION: Claremont, California; Urbana, Illinois; Farina, Illinois.

ILLUSTRATIONS: Benjamin, Aliso **3**, *pl. 10–12.*

The above description is taken from the original diagnosis.

GRAIN PROCESSING CORPORATION
MUSCATINE, IOWA

BIBLIOGRAPHY

Baranetzky, J. 1872. Entwicklungsgeschichte des *Gymnoascus reessii.* Bot. Zeit. **30**: 145–160.

Benjamin, C. R. 1955. Ascocarps of *Aspergillus* and *Penicillium.* Mycologia **47**: 669–687.

Benjamin, R. K. 1956. A new genus of Gymnoascaceae with a review of the other genera. Aliso **3**: 301–328.

Berkeley, M. J. 1838. Notices of British fungi. Ann. Nat. Hist. **1**: 257–264.

——. 1860. Outlines of British Fungology. Lovell Reeve, London. 442 pp.

—— **and C. E. Broome.** 1875. Notices of British fungi II. Ann. Mag. Nat. Hist. IV **15**: 28–41.

Beyma thoe Kingma, F. H. van. 1942. Beschreibung einiger neuer Pilzarten aus dem Centraalbureau voor Schimmelcultures, Baarn (Netherland). VII. Ant. van Leeuwenhoek Jour. Microbiol. Serol. **8**: 105–122.

Brefeld, O. 1891. Untersuchungen aus dem Gesamtgebiete der Mykologie **10**: 158–160.

Cain, R. F. 1956. Studies on soil fungi. *Saturnomyces* a new genus of the Aspergillaceae. Can. Jour. Bot. **34**: 135–141.

Castellani, A. 1937. A preliminary report on two pathogenic fungi: *Trichophyton Dankaliense* n. sp.; *Sporotrichum Anglicum* n. sp. Jour. Trop. Med. Hyg. **40**: 313–318.

Chalmers, A. J. and A. Marshall. 1914. The systematic position of the genus *Trichophyton* Malmsten 1845. Jour. Trop. Med. Hyg. **17**: 289–291.

Clements, F. C. and C. L. Shear. 1931. The Genera of Fungi. H. W. Wilson, New York. I–IV, 496 pp.

Cooke, M. C. 1871. Handbook of British Fungi. *2* vols., MacMillan, New York. 981 pp.

Currey, F. 1854. On two new fungi. Quart. Jour. Micr. Sci. **2**: 240–242.

Dale, E. 1903. Observations on Gymnoascaceae. Ann. Bot. **17**: 151–196.

Dangeard, P. A. 1907. L'origine du périthèce chez les ascomycètes. Botaniste **10**: 85–105.

Datta, S. 1932. A new fungus, *Eidamella actoni*, parasitic to the dog. Indian Vet. Jour. **8**: (cited from Rev. Appl. Mycol. **11**: 716. 1932, and Gregory, **1935**).

DeLamater, E. D. 1937a. Crozier formation in Gymnoascaceae: a preliminary report. Mycologia **29**: 187–198.

——. 1937b. *Eidamella spinosa* (Matruchot and Dassonville) refound. Mycologia **29**: 572–582.

Dodge, C. W. 1935. Medical Mycology. Mosby, St. Louis. 900 pp.

Eidam, E. 1880. Beiträge zur kenntnis der Gymnoascaceen. Cohn Beitr. Biol. Pfl. **3**: 267–305.

——. 1886. Untersuchungen über die Familie der Gymnoascaceen. Ber. Bot. Sect. Schles. Ges. **1886**: 160–165.

Emmons, C. W. 1935. The ascocarps in species of *Penicillium*. Mycologia **27**: 128–150.

Farrow, W. M. 1954. Tropical soil fungi. Mycologia **46**: 632–646.

Ferro, G. 1907. Osservazioni critiche antorno ad alcune specie conservate nell'erbario micologico P. A. Saccardo riferite al gen. *Myxotrichum* Kunze. Nuovo Gior. Bot. Ital. II **14**: 221–234.

Fischer, A. 1897. Engler & Prantl, Die Natur. Pflanzenf. **1** (1): 294.

Gäumann, E. 1949. Die Pilze. Verlag Birkhäuser, Basel. 382 pp.

Gilman, J. C. 1957. A Manual of Soil Fungi. 2 ed. Iowa State College Press, Ames, Iowa. I–XIV, 450 pp.

Gregory, P. H. 1935. The dermatophytes. Biol. Rev. Cambridge Phil. Soc. **10**: 208–233.

Grigorakis, L. 1925. Recherches sur les dermatophytes. Ann. Sci. Nat. Bot. X **7**: 165–444.

——. 1929. Dermatophytes et Dermatomycoses. Ann. Derm. et Syph. VI **10** (1): 18–68.

Grove, W. B. 1922. New or noteworthy fungi. Jour. Bot. **60**: 170–171.

Guilliermond, A. 1903. Contribution à l'étude de l'épiplasme des ascomycètes et recherches sur les corpuscules métachromatiques des Champignons. Ann. Myc. **1**: 201–215.

Höhnel, F. von. 1905. Mycologische Fragmente. Ann. Myc. **3**: 402–409.

Hotson, J. W. 1936. A new species of *Arachniotus*. Mycologia **28**: 497–502.

Karow, E. O. and J. W. Foster. 1944. An antibiotic substance from species of *Gymnoascus* and *Penicillium*. Science II **99**: 265–266.

Kuehn, H. H. 1954. Comparative Morphology of the Gymnoascaceae. Doctoral Dissertation, University of Illinois. Pp. 1–130.

——. 1955a. Observations on Gymnoascaceae. I. *Myxotrichum uncinatum* and a new species of *Myxotrichum*. Mycologia **47**: 533–545.

——. 1955b. Observations on Gymnoascaceae. II. Two new species of *Myxotrichum*. Mycologia **47**: 878–890.

——. 1956. Observations on Gymnoascaceae. III. Developmental morphology of *Gymnoascus reessii*, a new species of *Gymnoascus* and *Eidamella deflexa*. Mycologia **48**: 805–820.

——. 1957a. Observations on Gymnoascaceae. IV. A new species of *Arachniotus* and a reconsideration of *Arachniotus trisporus*. Mycologia **49**: 55–67.

——. 1957b. Observations on Gymnoascaceae. V. Developmental morphology of two species representing a new genus of the Gymnoascaceae. Mycologia **49**: 694–706.

Kunze, G. 1823. Einige neue oder verkannten Pilzgattungen und Arten. X. *Myxotrichum*. Myk. Hefte **2**: 108–110.

Marsh, R. W. 1926. Additional records of *Ctenomyces serratus* Eidam. Brit. Myc. Soc. Trans. **10**: 314–316.

Massee, G. 1895. British fungus flora IV. George Bell & Sons, London. 522 pp.

—— **and E. S. Salmon.** 1902. Researches on coprophilous fungi. II. Ann. Bot. **16**: 57–93.

Matruchot, L. and C. Dassonville. 1899a. Sur le champignon de herpes (*Trichophyton*) et les formes voisines, et sur la classification des ascomycètes. Bull. Soc. Myc. Fr. **15**: 240–253.

—— **and** ——. 1899b. Sur le *Ctenomyces serratus* Eidam comparé aux champignons des teignes. Bull. Soc. Myc. Fr. **15**: 305–310.

—— **and** ——. 1900. Sur une forme de reproduction d'order élevé chez les *Trichophyton*. Bull. Soc. Myc. Fr. **16**: 201–208.

—— **and** ——. 1901. *Eidamella spinosa*, dermatophyte préduisant des périthèces. Bull. Soc. Myc. Fr. **17**: 123–132.

—— **and** ——. 1902. *Eidamella spinosa*, dermatophyte préduisant des périthèces. Rev. Myc. **24**: 25–26.

Milochevitch, S. 1935. Une nouvelle espèce pathogène de *Ctenomyces*, *Ctenomyces bossae* n. sp. Ann. Parasitol. Hum. Comp. **13**: 559–567.

Nannizzi, A. 1926. Richerche sui rapporti morfologici e biologici tra Gymnoascaceae e Dermatomiceti. Ann. Myc. **24**: 85–129.

——. 1927. Ricerche sull'origin saprofitica dei funghi delle tigne. II. *Gymnoascus gypseum* (n. sp.), forme ascophora del *Sabouraudites* (*Achorion*) *gypseum* (Bodin) Ota et Langeron. Atti R. Accad. Fisiocritici Siena X **2**: 89–97.

Olliver, M. and G. Smith. 1933. *Byssochlamys fulva* n. sp. Jour. Bot. **71**: 196–197.

Ota, M. and M. Langeron. 1923. Nouvelle classification des dermatophytes. Ann. Parasitol. Hum. Comp. **1**: 305–336.

Peyronel, B. 1914. Osservazioni critiche e sperimentali su alcune specie del genere *Dicyma* Boul. e sui loro stati ascofori. Ann. Myc. **12**: 459–470.

Pollacci, G. 1925. Nuove osservazioni sul cicle di sviluppo dei generi *Trichophyton*, *Achorion*, *Microsporon* e loro rapposti con forme saprofitiche. Bull. Soc. Bot. Ital. **1925**: 127–130.

——. 1932. Sulla posizione sistematica dei miceti delle tigne (Rivendicazioni). Atti Ist. Bot. R. Univ. Pavia IV **3**: 15–18.

Raillo, A. I. 1928. Microflora of the soil. Jour. All Russ. Congr. Bot., Leningrad. **1928**: 181–182.

——. 1929. Beiträge zur kenntnis der Boden-Pilze. Zentralblatt Bakt. Parasit. Inf. Abt. II **78**: 515–524.

Raper, K. B. and C. Thom. 1949. A Manual of the Penicillia. Williams and Wilkins, Baltimore. I–X, 875 pp.

Rehm, H. 1905. Ascomycetes exs. Fasc. 35. Ann. Myc. **3**: 409–417.

Rosenbaum, E. H. 1944. The development and systematic position of *Arachniotus trisporus*. Ann. Mo. Bot. Gard. **31**: 173–201.

Saccardo, P. A. Sylloge Fungorum. P. A. Saccardo, Pavia, Italy. 1886. **4**: 660; 1889. **8**: 824; 1895. **11**: 438; 1902. **16**: 805; 1906. **18**: 194–195; 1913. **22** (1): 596.

Saccas, A. 1950. Un nouveau champignon ascomycète, Gymnoascé, l'*Eidamella papyricola* nov. sp. Bull. Soc. Myc. Fr. **66**: 128–138.

Salmon, E. S. 1900. New or rare British fungi. Jour. Queckett Microscop. Club II **7**: 371–376.

Schroeter, J. 1893. Die Pilze Schlesiens. *In* Cohn Krypt.-Fl. Schles. **3** (*2*): 210–213.

Shear, C. L. 1902. *Arachniotus trachyspermus*, a new species of the Gymnoascaceae. Science II **16**: 138.

——. 1907. Cranberry diseases. U.S.D.A. Bur. Pl. Ind. Bul. No. 110.

——, **N. E. Stevens and H. F. Bain.** 1931. Fungous diseases of the cultivated cranberry. U.S.D.A. Tech. Bull. No. 258: 13.

Smith, A. L. 1896. Fungi new to Britain. Brit. Myc. Soc. Trans. **1**: 150–158.

——. 1903. New or critical microfungi. Jour. Bot. **41**: 257–260.

——. 1904. *Ctenomyces serratus:* a correction. Jour. Bot. **42**: 55.

—— **and C. Rea.** 1902a. Fungi new to Britain. Brit. Myc. Soc. Trans. **2**: 31–40.

—— **and** ——. 1902b. Fungi new to Britain. Brit. Myc. Soc. Trans. **2**: 59–67.

Subramanian, C. V. 1952. Fungi isolated and recorded from Indian soils. Jour. Madras Univ. **22B**: 206–222.

Taber, W. A. 1951. Fungi of two forest soils of Johnson county. Proc. Iowa Acad. Sci. **58**: 209–214.

Tate, P. 1929. The dermatophytes or ringworm fungi. Biol. Rev. **4**: 41–75.

Thower, L. B. 1954. The rhizosphere effect shown by some Victorian health-land plants. Austr. Jour. Bot. **2**: 246–267.

Vailionis, L. 1936. Une nouvelle espèce de *Gymnoascus*. Vyt. Didziojo Univ. Mat. Gamtos Fak. Darbai **11**: 115–121.

Van Tieghem, P. 1877. Sur le développement de quelques Ascomycètes. Bull. Soc. Bot. Fr. **24**: 157–161.

Warcup, J. N. 1951. Studies on the growth of Basidiomycetes in soil. Ann. Bot. Lond. II **15**: 305–317.

West, B. and L. Ajello. 1956. The occurrence of *Arachniotus citrinus* in soil. Mycologia **48**: 163–165.

Westling, R. 1909. *Byssochlamys nivea*, en foreningslank mellam familjerna Gymnoascaceae och Endomycetaceae. Svensk. Bot. Tidskr. **3**: 125–137.

Wilenczyk, A. 1927. Sur le formation d'asques dans le *Trichophyton*. Ann. Inst. Pasteur **41**: 1351.

——. 1928a. Formation des asques dans les cultures de *Trichophyton*. Compt. Rend. Soc. Biol. Paris **98**: 70.

——. 1928b. La formation d'asques chez *Achorion schoenleini* (favus). Compt. Rend. Soc. Biol. Paris **98**: 1049.

——. 1928c. Sur la présence d'asques dans les squames des éruptions de mycoses (favus and trichophyta) et de seborhée. Compt. Rend. Soc. Biol. Paris **99**: 1027.

——. 1929a. Sur la formation des asques murs dans les teignes. Compt. Rend. Soc. Biol. Paris **100**: 1145.

——. 1929b. Sur la formation des asques chez l'*Epidermophyton*. Compt. Rend. Soc. Biol. Paris **101**: 593.

——. 1929c. Étude expérimentale des asques de champignons parasites des cheveux. Compt. Rend. Soc. Biol. Paris **102**: 113.

Winter, G. 1887. *In* Rabenhorst Krypt.-Fl. Deutschl. **1** (*2*): 2, 3, 15–17.

NOTES AND BRIEF ARTICLES

The spores of Endogone and Melanogaster in the digestive tracts of rodents

In 1956, the writer reported [1] finding the spores of the mucorine fungus *Endogone fasciculata* Thaxt. in the stomach contents of white-footed mice, meadow voles and a jumping mouse trapped in Saskatchewan. This fungus was also isolated for the first time from a variety of soil samples collected in Saskatchewan and northern Manitoba. These findings extended the previously known distribution of *Endogone* in Canada [2] another 450 miles to the east of the Alberta border.

Dowding had suggested that the sporocarps of *Endogone* may be stored by burrowing rodents and used by them for food. Since the writer also found the spores of *Endogone* in the digestive tracts of rodents and in soil in and around their burrows, he felt that this tended to confirm Dowding's suggestion that the sporocarps are eaten by the rodents. However, a survey conducted during the summer of 1956 has revealed an interesting observation which may have a bearing on this matter. During this survey the stomach contents of rodents trapped in various areas in Saskatchewan, Manitoba and northern Ontario, were examined for the spores of *Endogone* and other fungi. A large number of soil samples were also taken from these as well as other areas. Examination of these specimens showed that in the majority of instances the spores of *Endogone* were associated with numerous dark fungal spores which have been identified tentatively as the basidiospores of *Melanogaster variegatus* (Vitt.) Tul. (Fig. 1). This association was observed in specimens taken from white-footed mice (*Peromyscus maniculatus*) and meadow voles (*Microtus pennsylvanicus*) trapped in areas as widely separated as Saskatoon, Saskatchewan and Riding Mountain National Park, Manitoba. The same association of spores was also found in soil samples taken from widely separated sites in Saskatchewan (Saskatoon, Beaver Creek, Watrous), Manitoba (Riding Mountain National Park, Winnipeg, Gimli) and Kenora, Ontario (Fig. 2). Re-examination of permanent slides made during the 1955 survey revealed

[1] Bakerspigel, A. Endogone in Saskatchewan rodents. Amer. Jour. Bot. **43**: 471–475, 1956.

[2] Dowding, E. S. Endogone in Canadian rodents. Mycologia **47**: 51–57, 1955.

FIG. 1. Basidiospores of *M. variegatus*, × 1200. From stomach contents of a meadow vole, Saskatoon.

FIG. 2. A chlamydospore of *E. fasciculata*, × 1200. From soil, Kenora, Ontario. The basidiospores of *M. variegatus* are out of focus.

FIG. 3. A chlamydospore of *E. fasciculata* and the basidiospores of *M. variegatus*, × 1200. Collected near Dauphin, Manitoba.

a similar association of spores in the stomach contents of several white-footed deer mice and meadow voles trapped that summer in Saskatchewan.

Field mice and other rodents are known to feed upon a variety of fungi. It is also known that some mucors not only parasitize other members of the Mucorales but also parasitize the fruiting bodies of larger fungi.[3] In the light of the observations recorded above it is suggested that *Endogone* parasitizes *Melanogaster* and that in these instances the finding of *Endogone* spores in rodent stomach contents is probably due to their having been ingested together with *Melanogaster*. The writer has to this date collected one melanogaster near Dauphin, Manitoba, from which the spores of *E. fasciculata* have been isolated (FIG. 3).

I am grateful to Dr. D. B. O. Savile, Science Service, Ottawa, Canada, for his assistance in identifying the basidiospores of *Melanogaster variegatus*.—A. BAKERSPIGEL, Department of Bacteriology and Immunology, University of Western Ontario, London, Canada.

ON PREPARING FLESHY FUNGI FOR THE HERBARIUM

Preparing fleshy fungi for the mycological herbarium constitutes an indispensable part of the routine involved in maintaining a permanent collection. As practised today throughout the United States and in many herbaria elsewhere, the procedure most commonly followed is to dry the freshly collected mushrooms thoroughly and deposit them in labelled boxes of appropriate size for future use. As anyone familiar with this scheme knows, such boxes often occupy a disproportinate amount of space in a standard (and expensive) herbarium cabinet. It might therefore be of interest to report on another method of preparation, more exacting and somewhat more time-consuming, but deserving consideration because of the space-saving and possibly other benefits it offers.

The technique to be outlined enables the user to mount pressed specimens of mushrooms on herbarium sheets, as is done with higher plants. The mounted specimens, enclosed in packets, retain very closely their original size and shape and are at least aesthetically more desirable (FIG. 1) than mushrooms dried in the conventional manner. Although

[3] Hawker, L. E. Physiology of Fungi. University of London Press, London, 1950.

the time required for their preparation is greater, it is not excessively so, especially after a little skill has been acquired in the necessary manipulations. One disadvantage that might be pointed out in adopting the alternate method to be suggested is that fragments of the specimens must be detached if microscopic characteristics need to be studied, but by enclosing additional dried material in the packet this objection is overcome. Moreover, the great economy of space attained and the vastly improved appearance of the collections are notable advantages over the older method. The latter point should not be underestimated, since it

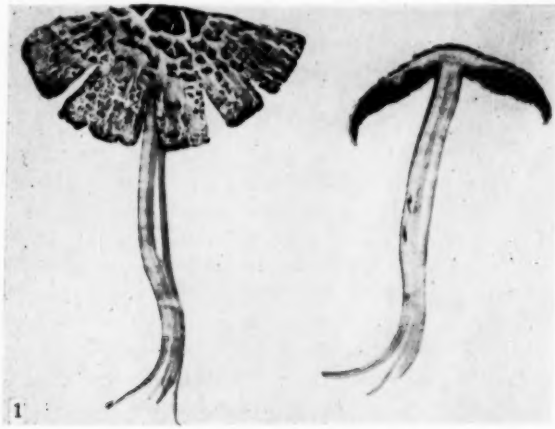


FIG. 1. *Panaeolus solidipes* Pk. prepared according to the method of G. Bohus. × ½.

is well known that many mushrooms, on drying, bear little resemblance to their original condition, thus making their identification more laborious.

During the course of an exchange of fungi with Dr. Gabriel Bohus, mycologist of the Museum of Natural History in Budapest, I have received over 100 specimens of mushrooms mounted on sheets. In response to my request for information concerning the technique of preparation, Dr. Bohus has generously provided me with the following explanation, which is a translation from the Hungarian of the pertinent parts of his letter.

Dissolve 40 g of gelatin in 200 cc of water and apply with a broad brush to sheets of a low-rag-content paper. A cheap grade of typewriterpaper is quite suitable. When the sheets have dried, place them

in folders beneath a weight in order to flatten them out. Using a straight razor, cut longitudinal sections about 1–1.5 mm thick through the central part of the cap and stipe of a fresh mushroom, in a plane parallel to the gills. When the sections have been made (2 to 4 can be obtained, depending upon the diameter of the stipe), by cutting and scraping remove the gills from the halved cap, using a small spoon (the edge of which has been ground to sharpness) or a single-edged razor blade. When this operation is complete, the delamellated cap should be about 1–1.5 mm thick. The flesh of the remaining part of the stipe is then scraped out to about the same thickness. Place the prepared caps and stipes on gelatin-coated paper and press, using the usual blotters, newspapers and corrugated cardboards. Large, fleshy forms should have little pressure applied to them at first, while less fleshy ones may be pressed more vigorously from the start. With viscid mushrooms, lay a fine-textured cloth such as chiffon, poplin or batiste directly over the specimen in order to prevent the newspaper from adhering. If, however, the fungus sticks to the cloth, it may be easily removed by the application of a few drops of water to the adhering part. Blotters should be changed as often as required until the mushrooms are quite dry. After drying, they may be cut out when convenient and pasted on herbarium-grade paper of the desired size. The stipes are attached first (white paste produces very satisfactory results), then the caps, arranging them to simulate their natural condition. In regions of high humidity, it is advisable, after the specimens are dry, to strip off the gelatin-coated paper by moistening in water and to mount them directly. This will tend to prevent molding. In any case, the chief function of the gelatin is to keep shrinkage at a minimum during the drying process. —BERNARD LOWY, Department of Botany, Bacteriology and Plant Pathology, Louisiana State University, Baton Rouge.

SYMBOLISM IN HETEROTHALLIC FUNGI

Blakeslee[1] originally applied the concept of heterothallism to two morphologically similar isolates which reacted sexually when paired. Since the two isolates showed no morphological differences, it was not possible to use the designations "male" and "female." To symbolize this situation, Blakeslee arbitrarily called one isolate "(+)." Then all

[1] Blakeslee, A. F. 1904. Sexual reproduction in the Mucorineae. Amer. Acad. Arts and Sci. Proc. **40**: 205–319.

isolates which mated with this were called "(−)," while all that did not mate were found to be also (+), in that they mated with those isolates already designated as (−). Inherent in this symbolism is the idea that relative sexuality does not exist, and that an isolate can be designated as (+) or (−) when tested with any arbitrarily defined tester strain.

Heterothallism is widespread in the fungi, and it is only natural that workers in different groups might use a different symbolism than Blakeslee's. Shear and Dodge [2] characterized the mating types in heterothallic *Neurospora* species as A and B. In Lindegren's [3] work on *Neurospora,* Blakeslee's original symbols were used, but Tatum and Beadle [4] later revised this and designated the mating types of A and a. In *Saccharomyces,* Lindegren [5] has used the symbols a and α to designate mating types. Catcheside [6] has used a uniform terminology for his discussion of mating types in all fungi, namely mt^+ and mt^-.

It seems to the writer that this diversity of symbolism is confusing, and a uniform system should be adopted. Several ground rules for such a uniform symbolism can be listed. 1: Mating type symbols should refer to the phenotypes of the isolates, rather than the genotypes, since it is generally not readily possible to characterize such a complex of physiological factors in terms of single genes. 2: The symbols should be easy to write on any typewriter. 3: The symbols should be easy to speak in oral communication. 4: If possible, priority should be respected.

On all of these counts, it would seem that Blakelee's original symbols would be the best. When letters are used, there is a tendency to equate them with genes. Especially the use of A and a seems to indicate dominant and recessive alleles, which situation does not exist. In addition, in oral usage, A and a must be spoken as "large A" and "small a." The symbols a and α are to be deplored because of the mixture of Roman and Greek letters, and the absence of α on most typewriters. A and B would

[2] Shear, C. L. and B. O. Dodge. 1927. Life histories and heterothallism of the red bread-mold fungi of the *Monilia sitophila* group. Jour. Agr. Res. **34**: 1019–1042.

[3] Lindegren, C. C. 1932. The genetics of *Neurospora.* II. Bull. Torrey Bot. Club **59**: 119–138.

[4] Tatum, E. L. and G. W. Beadle. 1942. Genetic control of biochemical reactions in *Neurospora:* an "aminobenzoicless" mutant. Proc. Natl. Acad. Sci. U. S. **28**: 234–243.

[5] Lindegren, C. C. 1949. The Yeast Cell, Its Genetics and Cytology. Educational Publishers, Inc., St. Louis. Pp. 10–12.

[6] Catcheside, D. G. 1951. The Genetics of Microorganisms. Pittman Publishing Corp., New York. P. 5.

seem to designate two different characters, and mt^+ and mt^- obviously refer to genes rather than isolates.

I therefore propose that the symbols (+) and (−) be used to designate isolates (not genes) which can be characterized as two different mating types. When the genetic aspects of mating type are worked out for any species, the genes can be designated by any desired letters without upsetting this symbolism.—THOMAS D. BROCK, Department of Biology, Western Reserve University, Cleveland 6, Ohio.

MEDICAL MYCOLOGY COURSES

The Laboratory Branch, Communicable Disease Center, U. S. Public Health Service, P. O. Box 185, Chamblee, Georgia, will offer laboratory methods courses in Medical Mycology—Cutaneous, Subcutaneous, and Systemic Fungi, from January 5–30, 1959; Study of Pulmonary Mycoses, February 9–20, 1959; and Diagnostic Methods in Veterinary Mycology, March 2–6, 1959. Information and application blanks from the Laboratory Branch.

REVIEWS

CHROMOTAXIA; CODE MYCOLOGIQUE ET PEDOLOGIQUE DES COULEURS, by Marcel Locquin. 65 pp., 1 pl., 6 fascicles of transparent color samples. Published by the author, 14, rue de Buffon, Paris 5me. 1958. 2500 fr.

Locquin's title can hardly fail to interest the mycologist who attempts to make use of color names in descriptive writings. His work is likely to prove disappointing in use, though most ingenious in conception and execution.

The set of basic color samples consists of 2.0 × 1.5 cm pieces of colored gelatin filters attached to the distal ends of double plastic strips (9 × 2 cm) which are securely hinged and bracketed proximally by a colored dome fastener. Six fascicles each consist of six such samples of different relative densities (each labelled by its distinctive letter and number) and a strip with a white backing tab, on the support of which the hue name is written in full.

The colors are named Red, Yellow, Green, Cyan, Blue and Magenta and are respectively the R, Y, G, C, B and M filters of the Kodak color-compensating series. The work thus has taken advantage of the advanced color technology, standardization and production control of the Kodak company. It employs gelatin filters for which the spectral absorption characteristics are known and readily available (Eastman Kodak Co., **1957**). This should insure a reasonably constant supply for dependable replacements or re-issuings.

Within each set of a given "hue" the increase in relative density through the six stages alters both of the other two major psychological attributes of color, namely; "value" (lightness or darkness) and "chroma" (intensity or saturation). From one hue to another the effect of these steps on "value" is unavoidably vastly different. This characteristic imposes a limitation upon the rational placement of any given color within the color solid, a limitation which is shared, to a somewhat lesser degree, by the much-used system of Ridgway (**1912**), as pointed out by Hamly (**1949**). It is overcome most successfully by the Munsell standards (**1929**).

Reflectance from the glossy surface of the filters is disturbing and likely to be confusing to the user unless the specimen he is attempting

to match has a similar quality. It may be a personal idiosyncrasy, but combinations of these filters viewed by reflected light against the white backing tab appear to be unduly conditioned by the nature of the uppermost filter in the pile.

After some general remarks in the accompanying manual, Locquin proceeds to tabulate some "ambiguous color terms," references and a four-language glossary of terms used to describe qualities of color. The body of his booklet (pp. 29–63) consists of tables of color names in French, English, German and Latin keyed to combinations of the available color filters. These tables are logically arranged with related colors brought together under such headings as COULEURS PURES, LES ORANGES LAVÉS DE BLANC, LES ROUGES RABATTUS, etc. That this arrangement cannot be more systematic is no fault of the author, for here he is clearly limited by the methods employed.

One must question, as has been done with much service to mycology by Dade (**1943**), the advisability of introducing a large color vocabulary to descriptive writings. The introduction of such terms as *lignyzon*, *phoeniceus* or *thallassicus* into Latin diagnoses would seem to serve no useful purpose.

Failure to refer to the joint work of the I.S.C.C. and the N.B.S. under the aegis of Kelly and Judd (**1955**) appears to be a major and significant omission.

For the mycologist who finds himself without a set of color standards, the work under review, despite its attractively (and deceptively) low price tag offers no solution. As a prominent British authority has pointed out to me in correspondence, neither the impractical and exasperating juggling with the fans of filters nor the author's plagiarism of Saccardo's title adds stature to Locquin's device.

As an alternative to this and the no longer available Ridgway *Standards*, a mycologist has a choice between the screen-printed *Colour Atlas* by Villalobos (**1947**) and the recently devised Mycologists' Color Kit of the Munsell Color Co. (**1958**). The former is well designed and executed, considering the methods used. The introduction of twelve hue names with first and second order intermediates designated by two- and three-letter codes respectively will prove more cumbersome to the user than the 100-hue circuit of Munsell built around five well-accepted hue names. Fuller comparisons of the various systems available in 1952 will be found in Judd (**1952**).

The Mycologists' Kit is an assembly of stock papers ($\frac{3}{8}$" x $\frac{1}{2}$") of Munsell Colors chosen to include, in addition to the chips present in the Students' Set or the Pocket Edition of the Munsell Book of Color

(Munsell **1929, 1942**), all of those available colors deemed likely to prove useful in descriptive mycology. A description of this kit and suggestions for its assembly are available from the company upon request. Those who possess the Pocket Edition or Students' Set already may purchase the 'Mycologists' Extension Chips' separately, and thus supplement the basic work with much-needed low chroma and high value colors.

The descriptive mycologist can hardly afford to be without the papers by Dade (**1949**) and Kelly and Judd (**1955**) as necessary adjuncts in his use of either Ridgway or Munsell standards.—WM. IRWIN ILLMAN, Dept. of Biology, Carleton University, Ottawa, Ontario.

Dade, H. A. 1949. Colour terminology in biology. 2nd Edition. Commonwealth Myc. Inst., Kew.

Eastman Kodak Co. 1957. Kodak Wratten Filters, pp. 64–75. Rochester, N. Y.

Hamly, D. H. 1949. The Ridgway Color Standards with a Munsell Notation Key. Jour. Opt. Soc. Am. **39**: 592–599.

Judd, D. B. 1952. Color in business, science and industry. Wiley, New York.

Kelly, K. L. and Judd, Deane B. 1955. I.S.C.C.–N.B.S. method of designating colors and dictionary of color names. 158 pp. Washington, D. C.

Munsell, A. H. 1929 & 1942. Munsell Book of Color. Baltimore, Md.

Munsell Color Co. 1958. Mycologists' Color Kit with descriptive literature. 10 E. Franklin St., Baltimore 2, Md.

Ridgway, R. 1912. Color Standards and Color Nomenclature. 43 pp. Washington, D. C.

Villalobos-Dominguez, C. and Villalobos, J. 1947. Colour Atlas. El Ateneo, Buenos Aires, Argentina.

MUSHROOMS RUSSIA AND HISTORY, by Valentina Pavlovna Wasson and R. Gordon Wasson, 2 vol.; XXI + 433 pp.; 82 pl., 28 fig., 3 vignettes, partly colored; 1 chart. Pantheon Books, New York, 1957. $125.00.

As a mycologist interested in the impressions fungi in general and mushrooms in particular have made on the human race since the dawn of history, I have never encountered a work both as interesting and as attractively written as "Mushrooms Russia and History" by the Wassons. It is sheer pleasure to follow their lines of interest as they bring these into focus and to observe the cleverness with which they present their own views on many questions. Since the outstanding good points of this publication have been reviewed by so many, and in particular in MYCOLOGIA (**50**: 147–148) by D. P. Rogers, to dwell at length on them again would be mere repetition.

My reason for contributing a second review is that certain mycological items overlooked by previous reviewers need to be pointed out

for the benefit of mycologists in general. Hence the fact that I am considering some shortcomings of the publication is by no means to be construed as a condemnation of the work as a whole. It is truly a monumental piece of work, and, as is true of nearly all such works, by nature it has much in it that is controversial. It is abundantly clear from the text that the authors were well aware of this. However, as an agaricologist I was shocked by a number of errors that are serious to one interested in the accurate identification of mushrooms.

The worst error is in the name for the beautifully reproduced color plate (XXXV) of a painting by Fabre designated as *Amanita muscaria*. I have located nothing in the text that explains why this illustration was used. *A. muscaria* does not have a free-margined, saccate volva of the *A. phalloides* or *A. caesarea* type, such as is shown in this illustration. Fabre may have painted what he saw on the specimen at hand, and, if so, in this reviewer's opinion he more than likely had an aberrant collection of *A. caesarea*. No matter from what point of view you consider this painting, there is no satisfactory way of explaining this aberrant character as being present in *A. muscaria*. Allowing such a misleading illustration as this to be published for a well known and common species is a serious error, and the authors' mycological advisor, Roger Heim, should not have allowed his friends to use it. For one thing, having such an error so prominently displayed has the effect of taking all the punch out of the Wassons' criticism of professional mycologists who have also made mistakes. As far as the Wassons' criticism of the "learned doctors"—as Lloyd called them—is concerned, a great deal of it has some justification; the mycological world, however, actually had its fill of this type of writing from the pen of C. G. Lloyd early in this century. This became more apparent as it was found that Lloyd made practically all the mistakes he so vigorously derided the "learned doctors" for making. The Wassons' style is, of course, much more refined than Lloyd's, but the result is the same. People in glass houses should not throw stones—at least not large ones!

The second grave error is in the name for the painting designated as *Lactarius piperatus* (Pl. XXXI). As any amateur mycologist who has studied these large white *Lactarii* knows, the outstanding field characters within the group are the narrow, crowded, repeatedly forked gills. In this day and age it is an offense to illustrate this species in a work on mushrooms and not show these characters. But what do we see in Fabre's painting? As an agaricologist I see a most beautiful illustration of mature fruiting bodies that have the macroscopic characters of *L. vellereus*. The excellent illustration of *L. vellereus* in Wakefield and

Dennis (Pl. 37, fig. 3) should be compared with Fabre's painting. As far as I can see there simply is no defense for labeling Fabre's painting *L. piperatus*. As people who collect wild mushrooms to eat, the Wassons themselves should have become aware of both errors from a glance at the paintings.

I should also like to question a statement they make (p. 11) about *Boletus aereus*, which they call the *borovik* and characterize as a bolete occurring under pine. This situation is peculiar because Roger Heim in his new publication *Les Champignons d'Europe* characterizes it as growing under oak, which is exactly what Singer has claimed; and yet the Wassons chose to doubt Singer's statement, and, in contrast, published one that is actually contradicted by both Singer and their own advisor on matters mycological. Personally, I have always used the name *Boletus variipes* for this species since it is a name which applies to American material and for which we have a type specimen, and thus have avoided entering the discussion of the identity of *B. aereus*.

One gets the impression from reading vol. I that the Wassons are too enthusiastic about recommending just mushrooms in general to the public, and the two errors pointed out above go a long way to substantiate this impression. The authors belittle the severity of mushroom poisoning when they state of *A. muscaria* (p. 190), "There is a general belief that the victim who eats it dies; yet we believe that this murderous accusation is unsupported by a single trustworthy case history." As a mushroom specialist I have never encountered a true belief that *A. muscaria* was deadly poisonous, but I challenge the statement that it has never caused the death of a human being in a "trustworthy case." That of the fatal poisoning of the Count de Vecchii in November 1897, which is cited by Fischer in his discussions of mushroom poisoning by *A. muscaria* (in Kauffman's *Agaricaceae of Michigan*, p. 838) is acceptable to me. Since the Wassons have undoubtedly also read this case history they presumably regard it as not trustworthy. Certainly they are under obligation to the public to clarify their statement. As to the fly-killing ability of *A. muscaria*, I have one pertinent comment. Dr. Ernst A. Bessey, recently deceased, told me in detail how, when he was studying in Germany as a young man, a group of students including himself performed the experiment of the flies with *A. muscaria*. According to Dr. Bessey some of the flies died, but most recovered. He also stated that at East Lansing he tried the same experiment and found the Michigan variety of *A. muscaria* less potent. I see no valid reason for not accepting the fact that *A. muscaria* has been used as a "fly killer" even if it is not as efficient as DDT! Since all mushrooms—*i.e.*, these plants as a group—

were at one time associated with the supernatural, perhaps for varied reasons, it seems pointless now to argue over which meaning came first.

One gets the impression from reading the Wassons' two-volume work that they measure the competency of an agaricologist by the amount of mushrooms he eats. This amuses me somewhat because I happen to know all the details surrounding one of their statements. Since it concerns me (which is why I know all the details) I hope my readers will pardon this personal diversion. In vol. 1, chap. II, p. 19, the Wassons state: "In a recent elaborate American work on mushrooms the author [referring to *Mushrooms in their Natural Habitats*] brings himself to write a chapter on 'mycophagy,' but confesses that he himself almost never lets mushrooms enter his mouth!" The Wassons did not see fit to try to ascertain why I take the attitude I do—which is not at all accurately expressed by the wording of their text! Just for the record I should like to state that after chewing material of about 60,000 collections of wild mushrooms in the raw state to record any flavor that may be taxonomically or otherwise significant, I think I can justifiably be excused from having them again at meal time! The humorous aspect of this unimportant situation, of course, was created by the way the Wassons worded their statement—which makes it completely false. One can't help but wonder how many other comparable situations exist in the book, if only the simple facts were known and presented in an unbiased manner.—ALEXANDER H. SMITH.

MANUSCRIPT

Publication in MYCOLOGIA is ordinarily restricted to those who have been members in good standing of the Mycological Society of America for over a year immediately preceding submission of manuscript. Exceptions to this regulation require a favorable vote by a majority of the Editorial Board. When a paper has two or more authors, the person submitting the paper is expected to be a member.

Papers should be submitted in duplicate, typewritten and *double-spaced throughout*, to any member of the Editorial Board. When papers are not submitted in proper form, it may be necessary to return them to authors. They will be published in approximate order of their acceptance, except for the address of the retiring President and papers whose cost of publication is paid by the authors, the latter run as excess pagination.

All illustrations should be numbered consecutively throughout a paper, using arabic numbers and small letters for subdivisions, e.g., Fig. 1, a etc. This does not mean that all figures grouped for convenience on a single page need have a single number. Figures should be prepared so that, when reduced, the width will not exceed 4 inches, and should be short enough to permit the insertion of the legend beneath the figures. Each article will be restricted to twenty pages, including illustrations, except when authors submit only one paper in two or three years of membership, in which case the restriction will be thirty and forty pages respectively. Ruled tabular matter is counted double. Should an author wish to publish additional pages in one article he may do so by paying for the excess pages at current rates.

Citations of literature should be *double-spaced*, arranged in alphabetical order and cited by numbers or dates. In citing papers with two or more authors, only the first author should have the initials after the surname. The address of the author should appear at the end of the text, before the bibliography.

Each article will be restricted to two pages of half-tone illustrations or three of zinc line-engravings for each article, the total cost not to exceed $25. If figures are mounted on board, the cost of routing may be such as to restrict the space allowance substantially. Should the cost of cuts exceed $25, the author will be asked to pay the excess.

To comply with the International Rules, it is recommended that contributors furnish *brief* Latin diagnoses of all new species and genera when their manuscript is submitted for publication.

PRICE LIST OF REPRINTS

	4pp. 1 to 4	8pp. 5 to 8	12pp. 9 to 12	16pp. 13 to 16	20pp. 17 to 20	24pp. 21 to 24	28pp. 25 to 28	32pp. 29 to 32
50 Copies	$4.40	$7.00	$11.00	$11.45	$15.00	$17.15	$19.80	$21.15
100 Copies	5.25	8.35	12.75	14.10	18.00	21.15	24.65	26.45
Additional Copies per C...	1.75	2.65	4.40	5.30	6.00	8.00	9.70	10.60

For 500 copies deduct 5%; for 1000 copies or more deduct 10%.

Covers: For first 50 covers, $4.80; additional $3.45 per C.

For more than 32 pages add cost per schedule to make total. Example: for 44 pages add cost for 32 pages and 12 pages.

Note: For any reprints requiring additional composition or changes, either in text or cover, an extra charge will be made.

LANCASTER PRESS, INC.
LANCASTER, PA.

Partial List of Publications of The New York Botanical Garden

---

Mycologia, bimonthly; devoted to fungi, including lichens; containing technical articles and news and notes of general interest. $8.50 a year; single copies $1.75 each.

Established by The New York Botanical Garden in 1909, in continuation of the Journal of Mycology, founded by W. A. Kellerman, J. B. Ellis, and B. M. Everhart in 1885. Edited by William Alphonso Murrill, 1909–1924. Edited by Fred Jay Seaver, 1924–1946; by Alexander H. Smith, 1946–1950. Edited by G. W. Martin, 1951–1957. Beginning with January, 1933, the official organ of the Mycological Society of America.

North American Flora. Descriptions of the wild plants of North America, including Greenland, the West Indies, and Central America. Planned to be completed in 34 volumes. Roy. 8vo. Each volume to consist of four or more parts. [Not offered in exchange.] Volumes 1–10 devoted to fungi.

Vol. 1, part 1, 1940. Myxomycetes. $7.25.

Vol. 2, part 1, 1937. Rhizochytriaceae, Monoblepharidaceae, Saprolegniaceae, Entomophthoraceae, Laboulbeniaceae. $2.00.

Vol. 3, part 1, 1910. Nectrioideae-Fimetariaceae. $2.00. (Out of print.)

Vol. 6, part 1, 1922. Phyllostictaceae (part). $3.00.

Vol. 7 (now complete), parts 1–15, 1906–1940. Ustilaginaceae-Nectrioideae. $2.00 per part. (Parts 1–5 out of print.)

Vol. 9 (now complete), parts 1–7, 1907–1916. Polyporaceae-Agaricaceae (part). $2.00 per part. (Parts 1–3 out of print.)

Vol. 10, part 1, 1914; parts 2 and 3, 1917; part 4, 1924; part 5, 1933. Agaricaceae (part). $2.00 per part.

Series II, part 1, 1956. Tuberales. $1.00.

The New Britton and Brown Illustrated Flora of the Northeastern United States and Adjacent Canada. By Henry A. Gleason. 3 volumes. List price $30.00 per set; shipping charge $0.50. Succeeds the Illustrated Flora by Nathaniel L. Britton and Addison Brown. Includes descriptions and drawings of the plant species, from ferns to orchids, which grow without cultivation in the area extending from the St. Lawrence River to Virginia and westward to Missouri and Minnesota.

The Garden Journal of The New York Botanical Garden. Bimonthly. Illustrated, containing news, book reviews, and non-technical articles on botany, exploration, and horticulture. Free to all members of the Garden. To others, 35 cents a copy, $2.00 a year. Now in its third volume. A continuation of the Journal of The New York Botanical Garden, fifty-one volumes.

Brittonia, quarterly; botanical papers; beginning in 1937, official journal of the American Society of Plant Taxonomists. $7.50 a year.

NEW YORK BOTANICAL GARDEN
Bronx Park, New York 58, N. Y.